EDICAL LIBRARY

JAN 22 1947

THIS ISSUE, WITH INDEX, COMPLETES VOLUME 39

Volume 39 Number 6

ENDOCRINOLOGY

DECEMBER, 1946

HOUSSAY, B. A., V. G. FOGLIA AND C. MARTÍNEZ

The Influence of the Thyroid on Alloxan and Pancreatic Diabetes in the Rat 361

DOUGHERTY, THOMAS F., AND ABRAHAM WHITE

Pituitary-Adrenal Cortical Control of Lymphocyte Structure and Function as Revealed by Experimental X-Radiation 370

ALBERT, S., AND C. P. LEBLOND

The Distribution of the Feulgen and 2,4-Dinitrophenylhydrazine Reactions in Normal, Castrated, Adrenalectomized and Hormonally Treated Rats 386

CRAFTS, ROGER C.

Effects of Hypophysectomy, Castration, and Testosterone Propionate on Hemopoiesis in the Adult Male Rat 401

CURLEY, F. J., AND W. L. AYCOCK

The Effect of Stilbestrol on Resistance to Experimental Poliomyelitis .. 414

FRAME, ELIZABETH G., AND JANE A. RUSSELL

The Effects of Insulin and Anterior Pituitary Extract on the Blood Amino Nitrogen in Eviscerated Rats 420

NOTES AND COMMENTS

FRANCKE, C.

The Effect of Gonadotrophins on Explanted Fragments of Ovaries from Immature Mice 430

NEW BOOK 432

The Human Frontier

INDEX 435

PUBLISHED MONTHLY FOR
THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

Annual Subscription $10.50 Single Copies $1.25

days and days of dysmenorrhea

The days lost because of dysmenorrhea mount up and can never be regained. Therefore, when a woman's calendar regularly prophesies inevitable painful menses, physiologic readjustment of the endocrine system is in order. This may be attained with PRANONE in the majority of patients whose dysmenorrhea is due to hormonal imbalance.[1] Premenstrual tension states, also of endocrine origin, are similarly benefited by PRANONE.

PRANONE *tablets*

PRANONE (anhydrohydroxy-progesterone) Tablets, exhibiting corpus luteum-like activity when administered orally, are available in 5 mg. and 10 mg. strengths; in boxes of 20, 40, 100 and 250 tablets.

[1]Harding, F. E.: Am. J. Obst. & Gynec. 50:56, 1945.
Trade-Mark PRANONE—Reg. U. S. Pat. Off.

SCHERING

Schering CORPORATION • BLOOMFIELD, NEW JERSEY
IN CANADA, SCHERING CORPORATION LIMITED, MONTREAL

ENDOCRINOLOGY

VOLUME 39 DECEMBER, 1946 NUMBER 6

THE INFLUENCE OF THE THYROID ON ALLOXAN AND PANCREATIC DIABETES IN THE RAT

B. A. HOUSSAY, V. G. FOGLIA, AND C. MARTÍNEZ

From the Instituto de Biología y Medicina Experimental, Costa Rica

BUENOS AIRES, ARGENTINA

THIS PAPER is a summary of the experiments carried out in the rat in order to determine: a) the effect of thyroidectomy on the diabetes produced by alloxan or by ample pancreatectomy (extirpation of 95% of the pancreas); b) the effect of hyperthyroidism on the same diabetic states. Some of these points have been studied by Houssay and Foglia, Prieto Díaz and Sara (1945), Houssay and Sara (1945), and Martínez (1945, 1946).

THE THYROID GLAND AND SUSCEPTIBILITY TO ALLOXAN

This was studied in white rats of the male sex ranging in weight from 100 to 200 grams, which were divided into 5 groups: a) the first group received powdered thyroid in a daily dose of 10 to 20 mg. per 100 grams of body weight for 20–29 days; b) the second group was treated similarly for 60 days; c) the third group was thyroidectomized 20 to 29 days before; d) the fourth group was treated with thiouracil in daily doses of 20 mg/100 grams body weight for 30 days; e) finally, the fifth group acted as control. All animals were fed the usual diet of our Institute which consists of: wheat 3 kg.; corn, 2 kg.; white bread, 5 kg.; milk, 14 litres, supplemented by yeast, lucern and cod liver oil.

Alloxan was administered intraperitoneally in one experiment (in doses of 100, 125, 140, 150, 175, 200, 300 and 350 mg./kg. body weight) (Table 1), and intravenously in another experiment (in doses of 10, 25, 35, 50, 75, 100, 125 and 150 mg./kg. body weight). (Table 2).

When alloxan was administered intraperitoneally it was observed that the thyroidectomized animals were more resistant to alloxan than

Received for publication August 23, 1946.

Copyright, 1947, by the Association for the Study of Internal Secretions.

the controls and the latter more resistant than the thyroid-treated animals. In fact, the determination of the L.D.$_{50}$ in the 3 cases gave the following results: 152 mg./kg. for the controls, 200 mg./kg. for the thyroidectomized animals and 145 mg./kg. for the thyroid-

TABLE 1. ALLOXAN SUSCEPTIBILITY (INTRAPERITONEAL) IN RATS: a) NORMALS, b) THYROIDECTOMIZED, c) TREATED WITH THYROID (10 MG./100 GM.) DURING 20 TO 29 DAYS

Alloxan doses mg./Kg.	Normals		Thyroidectomized		Hyperthyroid	
	Mortality	%	Mortality	%	Mortality	%
100	0/5	0.0	0/5	0.0	0/6	0.0
125	1/10	9.0	0/11	0.0	0/11	0.0
140	0/10	0.0	0/10	0.0	2/10	20.0
150	6/17	35.2	1/15	6.6	13/16	81.2
175	9/10	90.0	14/18	77.7	11/12	91.6
200	6/6	100.0	3/4	75.0	7/7	100.0
300	6/6	100.0	3/4	75.0	7/7	100.0
350	—	—	5/5	100.0	—	—
D.L.$_{50}$ Gaddum	152.0		200.0		145.0	

TABLE 2. ALLOXAN SUSCEPTIBILITY (INTRAVENOUS) IN WHITE RATS: a) NORMALS, b) THYROIDECTOMIZED, c) TREATED WITH THYROID (20 MG./100 GM.) DURING 20 TO 60 DAYS; TREATED WITH THIOURACIL (20 MG./100 GM.)*

Alloxan doses mg./Kg.	Percentage of Mortality				
	Normals	Thyroidectomized	Thyroid 20 days	Thyroid 60 days	Thiouracil 30 days
10	0	0	0	0	0
25	40	0	80	0	0
35	40	0	100	—	—
50	60	40	80	0	0
75	100	100	100	50	0
100	100	100	100	100	0
125	100	100	100	100	33.3
150	—	—	—	—	100

* Total amount of male animals: 180.

treated ones, using Gaddum's method. The determination of the L.D.$_{50}$ by other methods gave similar results.

When alloxan was administered intravenously, similar results were obtained, for the L.D.$_{50}$ as determined by the same procedure gave the following values: 49 mg./kg. for the controls; 74 mg./kg. for the thyroidectomized group and 24 mg./kg. for the thyroid-treated one.

In addition, Table 2 summarizes the results obtained after thyroid treatment had been prolonged for 60 days and when thiouracil in the daily dose of 20 mg./100 gms. had been administered during 30 days.

It was observed that when thyroid treatment is prolonged to 60 days the animal acquires a greater resistance to alloxan than the controls or those treated with thyroid for only 20 days. As far as

thiouracil is concerned it was possible to detect how this agent, whose primordial action is to inhibit the thyroid, confers to the animal an exceptional resistance to alloxan which is much superior to that observed in thyroidectomized animals.

Consequently, there is no doubt that resistance to alloxan, whatever the mode of administration may be, can be greatly modified. It is increased after thyroidectomy while hyperthyroid states bring about first a decrease in resistance after 20 days and later an increase above normal after 60 days. Moreover, it is noteworthy that treatment with thiouracil confers to the rat an exceptional resistance to alloxan. There was complete coincidence between the histological findings and the diabetogenic action of alloxan in all these experimental animals.

DIABETES AFTER SUBTOTAL PANCREATECTOMY AND HYPOTHYROIDISM

THYROIDECTOMY AND DIABETES

The appearance and evolution of diabetes was studied in male white rats as follows: a) Rats which were pancreatectomized (95%) a few days after total thyroidectomy. b) Rats which were pancreatectomized and thyroidectomized similarly and which received 4 mg. of powdered bovine thyroids daily. c) Rats which were pancreatectomized (95%) and later, when they were about to become diabetic, were thyroidectomized. d) Rats which were pancreatectomized (95%) and thyroidectomized once they were definitely diabetic. The results obtained were as follows:

Pancreatectomized and thyroidectomized simultaneously

Groups of male and female rats were studied, and the results obtained as to appearance and evolution of the diabetes are presented in Table 3. It can be observed that thyroidectomy had a marked preventive effect on the diabetes. In fact, after 1 year, 10 of 12 males (83%) and 10 of 13 females (77%) were alive and free of diabetes; of the non-thyroidectomized animals, 1 of 21 males (5%) and 9 out of 19 females (48%) were free of diabetes.

When diabetes did not develop in the thyroidectomized-pancreatectomized animals during the first three months, it did not appear subsequently (except in 1 case in 20).

Pancreatectomized-thyroidectomized animals treated with thyroid

Male and female rats averaging 100 grams in weight were thyroidectomized and pancreatectomized. Half of each group remained untreated while the others received 4 mg. of powdered thyroid per 100 grams of body weight during months.

This replacement therapy brought about an early appearance and a rapid evolution of the diabetes (Table 4). Of 12 males there were: diabetes during the first month, in 5 cases: during the second month,

in 3; during the third month, in 2. Of 12 females there were: in the first month, 4 cases of diabetes and in the second, 2 cases.

Correcting the thyroid insufficiency suppresses the protection conferred by thyroidectomy.

TABLE 3. DIABETES (GLYCEMIA: .150% OR MORE AFTER SEVEN HOURS OF FASTING) IN PANCREATECTOMIZED RATS (REMOVAL OF 95% OF PANCREAS) AND THYROIDECTOMIZED-PANCREATECTOMIZED (95%) RATS
Final Result after One Year of Observation

	Males		Females	
	Pancreatectomized	Pancreatectomized-thyroidectomized	Pancreatectomized	Pancreatectomized-thyroidectomized
Permanent diabetes and death	*20*	*2*	*9*	*3*
Transitory diabetes	1	6	1	0
No diabetes	0	4	9	10
Total of non-diabetic rats	*1*	*10*	*10*	*10*
Total	*21*	*12*	*19*	*13*
Percentage of diabetes	*95*	*16*	*47*	*23*

TABLE 4. DIABETIC AND NON-DIABETIC MALE RATS
After One Year of Observation

	Subtotally pancreatectomized (95%)	Subtotally pancreatectomized (95%) and thyroidectomized	Subtotally pancreatectomized (95%) and thyroidectomized. Treated with thyroid (4 mg./100 gm.)
Diabetic	20 (95%)	2 (16%)	12 (86%)
Non-diabetic	1* (5%)	10 (84%)	2 (14%)
Total	21 —	12 —	14 —

* Transitory diabetes.

Thyroidectomized 8 months after pancreatectomy

Thyroidectomy, when performed once the diabetic state is imminent with high fasting glycemia (above 0.12 mg./100 cc. but below 0.15 mg./100 cc.), did not prevent the development of the disease in 11 male rats, since it appeared the following month in 4, after 2 months in 5 and after 3 months in 2.

Thyroidectomy was carried out in 8 males which were definitely diabetic, with fasting glycemias between 0.2 and 0.35 mg./100 cc. There was no change observed in 1 rat, temporary improvement in 6, and complete cure in 1.

Diabetes and thiouracil treatment

Twenty-eight male rats with body weights ranging from 95 to 140 gms. were pancreatectomized (extirpation of 95% of the pancreas) and divided into 2 groups. One group of 15 animals received 20 mg./100 gm./day of powdered thiouracil by mouth; 3 months after the operation the dose was increased to 40 mg./100 gm./day. The remaining 13 animals were used as controls.

The results obtained are presented in table 5 where it can be seen that the appearance of diabetes was postponed in the rats treated with thiouracil, as compared with the controls. In fact, one and a half months after the operation 23% of the controls were diabetic with a corresponding 0% in the treated group. After two and a half months 84% of the controls and 40% of the treated animals were diabetic. Moreover when the dose of thiouracil was increased, the percentage of diabetics fell to 26 only to rise again later on.

TABLE 5. DIABETES IN SUBTOTALLY (95%) PANCREATECTOMIZED RATS TREATED OR NOT WITH THIOURACIL (20 TO 40 MG./100 GM./DAY). CARBOHYDRATES RICH DIET*

	Untreated		With thiouracil	
	Hyperglycemic		Hyperglycemic	
	Total	%	Total	%
1	0/13	0	0/15	0
1½	3/13	23	0/15	0
2½	11/13	84	6/15	40
3½	11/13	84	4/15†	26
4½	12/13	92	8/15	53
5½	12/13	92	9/15	60
6½	12/13	92	9/15	60

* The diet consisted of: Wheat flour, 35; Corn flour, 34; Casein, 20; Yeast, 5; Cod liver oil, 5; Salt mixture, 1. (McCollum and Davis).
† From this time: 40 mg./100 gm./day.

At the end of the experiment, that is six and a half months after pancreatectomy, 92% of the controls and only 60% of the treated animals were diabetic.

The rats which received thiouracil showed a 14% decrease in basal metabolism as compared to the controls, and a 29% decrease as compared to non-operated normal rats.

Consequently, it can be concluded that as much in the thyroidectomized animals as in those treated with thiouracil, there is a true protection with respect to the appearance of diabetes after pancreatecomy. The degree of protection was greater in the complete thyroid insufficiency brought about by thyroidectomy than in thyroid hypofunction as a result of thiouracil administration.

DIABETES AND HYPERTHYROIDISM

Thyroid administration from pancreatectomy on: 20 male white rats were pancreatectomized (removal of 95%) and treated with powdered

thyroid, the daily dose being 20 gm./100 gm. In table 6 where the percentage of diabetic animals is presented, it can be observed that the appearance of diabetes in the thyroid-treated rats preceded that in the controls, whereas 15 days after the operation 31.5% of the treated rats were diabetic only 11% of the controls were diabetic at the end of the first month.

Thyroid administration was temporarily suspended during 9 days causing the percentage of diabetics to drop to 26. Once re-established

TABLE 6. PERCENTAGE OF DIABETES IN SUBTOTALLY (95%) PANCREATECTOMIZED MALE RATS TREATED WITH THYROID POWDER (20 MG./100 GM./DAY) SOMETIME BEFORE THE BEGINNING OF DIABETES

Days after operation	Treated with thyroid		Untreated	
	Diabetic		Diabetic	
	Total	%	Total	%
15	6/19	31	0/18	0
26	5/19	26	2/18	11
60	3/17	17	14/18	78
130	2/15	13	15/17	88
No more treatment				
150	7/18	50	12/13	92
203	4/14	28	13/13	100
263	0/12	0	11/11	100
300	0/12	0	—	—

in the same dose, there was no further increase in diabetes while on the contrary the percentage of diabetics gradually fell to 13.3%.

Suspending the treatment indefinitely occasioned, during the following two months, a new rise in the percentage of diabetic animals, reaching 50% in the first and 57% in the second month, but later there was a definite decrease, until 10 months after the operation there were 12 rats which were free of diabetes.

As can be seen in table 6, the percentage of diabetics in the non-treated controls, kept increasing until there was 100% diabetes from 6 to 9 months after the operation.

Diabetes and thyroid treatment

The results described above led us to attempt thyroid treatment in incipient diabetes; this was carried out in 21 rats. Once partially pancreatectomized, we awaited until most of the animals presented fasting hyperglycemia above .150%. This was obtained in 90.4% of the animals, 44 days after the operation, at which point thyroid treatment was started, the daily dose being of 20 mg./100 gm.

The results obtained as shown in table 7, demonstrate that the administration of thyroid caused the percentage of diabetics to drop

rapidly to 0%. However, in spite of continuous treatment, diabetes reappeared until 33.3% of the animals were again diabetic, maintaining this percentage until the end of the experiment.

DISCUSSION

From the results obtained a number of facts can be arrived at. In the first place, and as far as the susceptibility to alloxan is concerned, it is evident that, whatever the administration route (intraperitoneal or intravenous), the thyroidectomized animals are more resistant than their controls. On the other hand, animals previously treated

TABLE 7. PERCENTAGE OF DIABETES AND MORTALITY IN WHITE RATS TREATED WITH THYROID (20 MG./100 GM./DAY) AT THE BEGINNING OF THE INCIPIENT DIABETES PERIOD

Days after operation	Diabetes		Mortality	
	Total	%	Total	%
38	17/21	81	0/21	0
44*	19/21	90.4	0/21	0
64	10/21	47.6	0/21	0
79	4/19	21	2/21	9.5
109	0/18	0	3/21	14
139	1/15	6.6	6/21	28.5
149	5/15	33.3	6/21	28.5
184	5/15	33.3	6/21	28.5

* Beginning of the thyroid treatment (20 mg./100 gm./day).

with thyroid during a short time (20 days) are much more susceptible. However, if thyroid is administered during 60 days the animals acquire a resistance above normal.

It is an interesting fact that rats previously treated with thiouracil show an extraordinary resistance to alloxan, much greater than that produced by thyroidectomy. It is possible that this resistance is in relation with the presence of sulfur, or better still with the sulphydryl group (-SH-) in the thiouracil molecule. Experiments in order to clarify this point are actually in progress.

The results obtained with respect to the influence of hypothyroidism on the evolution of diabetes after subtotal pancreatectomy demonstrated that: 1) thyroidectomy prevents the eventual appearance of diabetes but neither detains the course of incipient diabetes nor causes manifest diabetes to reverse its course; 2) hypothyroidism as brought about by thiouracil administration prevents the appearance of diabetes after subtotal pancreatectomy in 40% of the cases.

Consequently it seems evident that hypothyroidism exerts a certain degree of protection on the β cells which could be due to: a) a relative functional rest given to the islets since the lowered metabolism and rate of growth requires less insulin; b) a modification of the anatomical or chemical constitution of the islets, making them more

resistant to diabetogenic factors; c) increase in the production of insulin brought about by hypothyroidism.

Finally, if we consider the results related to hyperthyroidism and diabetes it can be seen that in pancreatectomized animals (95%) thyroid treatment accelerates the appearance of diabetes during the first 15 days and later causes it to disappear gradually. In incipient diabetes, as well, thyroid treatment is followed by the complete disappearance of the disease 2 months later.

Histological study of these animals, which was performed by Dr. G. Lucas, seems to demonstrate that thyroid administration in rats, in continuous form, would lead to an improvement or to the disappearance of diabetes due to a new formation of islets or to the enlargement of the existing ones in the portion of the pancreas which remain after operation. These results would be in accordance with those of Herring (1917) in rats and cats; Watrin and Florentin (1929) and 1931) in guinea pigs, and of Hess (1942) in the white rat.

Moreover, it is probable that if thyroid treatment causes an early and temporary burst of diabetes, this could be due to its extrapancreatic action which becomes apparent as: 1) higher postprandial hyperglucemia; b) toxic effect of thyroid treatment; c) decrease of pancreatic resistance.

It is interesting at this point to emphasize the difference which exists between the dog and the rat and note their totally different response to anterior pituitary extract; the latter produces in the rat increase of islet tissue and of the insulin content of the pancreas. On the other hand, in the dog it causes a lesion of β cells and diminution of insulin content and secretion.

With thyroid treatment in the rat we have observed a beneficial effect on diabetes after pancreatectomy while, on the contrary (in the pancreatectomized dog) this same treatment produces a diabetogenic effect with the appearance of destructive lesion in the β cells of the islets and a decrease in the emergency secretion of insulin.

CONCLUSIONS

Experiments designed to study the action of hypo and hyperthyroidism on diabetes from alloxan or following pancreatectomy in the rat, gave the following results:

a) Thyroidectomized animals showed a greater resistance to the diabetogenic and toxic actions of alloxan than the controls.

b) Resistance to alloxan was below normal 20 days after daily thyroid treatment and above normal 60 days after it.

c) Thiouracil treatment produced great resistance to alloxan more than thyroidectomy.

d) Thyroidectomy if performed simultaneously with subtotal pancreatectomy (95%) prevents the appearance of diabetes, but it has no effect on incipient or manifest diabetes.

e) Thiouracil treatment prevents the appearance of diabetes after pancreatectomy (95%) in a large percentage of animals.

f) Thyroid treatment in pancreatectomized rats (95%) caused an earlier appearance of diabetes during the first 15 days; later, however, this diabetic state gradually disappeared, never to reappear, in spite of continuous treatment. In the non-treated rats, on the contrary, the diabetes persisted or appeared in all animals.

g) Thyroid treatment of pancreatectomized rats in which diabetes was already incipient caused its complete disappearance. The non-treated controls all eventually became diabetic.

REFERENCES

HERRING, P. T.: *Quart J. Exper. Physiol.* 11: 231. 1917.
HESS, W. W.: *Anat. Rec.* 84: 526. 1942.
HOUSSAY, B. A., V. G. FOGLIA, H. PRIETO DÍAZ AND J. G. SARA: *Rev. Soc. argent de biol.* 21: 232. 1945.
HOUSSAY, B. A., and J. G. SARA: *Rev. Soc. argent de biol.* 21: 81. 1945.
MARTÍNEZ, C.: *Rev. Soc. argent. de biol.* 21: 254. 1945.
MARTÍNEZ, C.: *Rev. Soc. argent. de biol.* 22: 1946.
WATRIN, J., AND P. FLORENTIN: *Compt. rend. Soc. de biol.* 100: 111. 1929; 107: 372. 1931.

PITUITARY-ADRENAL CORTICAL CONTROL OF LYMPHOCYTE STRUCTURE AND FUNCTION AS REVEALED BY EXPERIMENTAL X-RADIATION[1]

THOMAS F. DOUGHERTY AND ABRAHAM WHITE

From the Departments of Anatomy and Physiological Chemistry, Yale University

NEW HAVEN, CONNECTICUT

A LYMPHOPENIA and profound alterations in lymphoid tissue were among the earliest described physiological effects of Roentgen radiation. Indeed, the lymphocyte has been shown by many investigators to be the most radiosensitive of body cells (Dunlap, 1942). The extensive literature describing the effects of radiation on lymphoid tissue has been summarized by Murphy (1926) and, more recently, by Selling and Osgood (1938).

The similarities between the described changes produced in lymphoid tissue by Roentgen radiation and those seen following a single injection of either pituitary adrenotrophic hormone or adrenal cortical preparations (Dougherty and White, 1945) suggested that pituitary-adrenal cortical secretion might be a normal, physiological mechanism concerned with certain of the physiological actions of X-rays. On this basis, the lymphocytolytic effect of X-rays would be similar to that of certain other agents, e.g., benzene and potassium arsenite, which affect lymphoid tissue only in the presence of the pituitary or the adrenal cortex (unpublished results). However, a study of various radiation doses (10, 25, 50, 100 and 200 r) demonstrated that whereas small doses of X-rays produced unmistakable alterations in lymphoid tissue structure and function of mice only in the presence of an intact pituitary-adrenal cortical mechanism, large degrees of radiation would produce similar effects even in the absence of the animals' adrenals (White and Dougherty, 1945a). These observations made possible the use of Roentgen radiation to examine further the hypothesis that certain normal structural changes in lymphoid tissue and their physiological consequences are under pituitary-adrenal cortical control and are due to the effect of adrenal cortical steroids on one of their end cells of action, the lymphocyte. If large radiation doses cause lymphocytolysis in the adrenalectomized animal, then there should occur under these experimental conditions all of the various physiological changes which have been

Received for publication September 9, 1946.

[1] This investigation was aided by grants from the Josiah Macy, Jr. Foundation and the Fluid Research Fund of the Yale University School of Medicine.

ascribed (White and Dougherty, 1946) to the lymphocytolic action of adrenal cortical secretion.

MATERIAL AND METHODS

Mice of both sexes, 60 to 80 days old and of the CBA strain (Strong) were used. The animals were reared from the time of weaning on Fox Chow fed *ad libitum*. In addition, a mixture consisting of 93 per cent calf meal, 3 per cent dried yeast powder, 1 per cent cod liver oil and 3 per cent wheat germ was available to the animals at all times. Male rats of the Sprague-Dawley strain and weighing approximately 200 gms. at the time of the experiments were used. These animals received Fox Chow *ad libitum*. Adrenalectomized mice were used one-day postoperative, after having received a single, subcutaneous injection of 0.025 mg. of desoxycorticosterone acetate in sesame oil (Schering) immediately following operation. Adrenalectomized rats received a solution of 0.7 per cent sodium chloride and 0.3 per cent sodium bicarbonate as drinking fluid and were used 3 days postoperatively. Hypophysectomized rats were used approximately 7 to 10 days after operation.

In all of the experiments, the animals subjected to X-rays received total body radiation. This was accomplished in the case of the mice by placing four to six animals in a covered, light cardboard box which, because of its shallowness, prevented the mice from shielding one another. Rats were radiated in pairs by being placed in an uncovered cardboard box. Radiation of all animals was delivered at the rate of 43 r per minute by using the following conditions: 125 Kv. 5 ma.; 30 cm. skin target distance; 3 mm. aluminum filter.[2]

Tail blood was used in the determination of the total white count, differential counts and whole blood specific gravity in both mice and rats. Differential counts were made on air-dried blood films stained with May-Grünwald Giemsa. Specific gravity of whole blood was determined by the falling drop method (Kagan, 1941). Following withdrawal of tail blood from mice for the specific gravity determination and cell counts, each mouse was decapitated with a sharp scissors. The free flowing blood was collected, allowed to clot and centrifuged. Total serum protein values were obtained by the falling-drop technique (Kagan, 1938).

At autopsy of the mice, the inguinal, axillary, mesenteric lymph nodes, thymus, spleen, Peyer's patches, and, in some mice, testis, liver and kidney tissue were taken for histological examination. All tissues were fixed in Bouin's solution, carried through the usual procedures, sectioned at 5 micra and stained with hematoxylin and eosin. In non-operated mice, adrenals were removed following radiation of the animals, fixed in 10 per cent formalin and stained with Sudan IV by the Herxheimer technique. Paired mouse adrenals from single animals were analyzed for total cholesterol by the method of Schoenheimer and Sperry as modified by Sperry (1938).

The electrophoretic studies were conducted in the Tiselius apparatus. Pooled blood obtained by heart puncture of groups of 12 mice was allowed to clot and the serum separated by centrifugation. Each sample of pooled serum was diluted with two volumes of the buffer used in the electrophoresis experiment. The diluted sera were dialyzed for 72 hours at 3° C. against 2

[2] Radiation of all animals was conducted by either Dr. E. A. Lawrence or Dr. H. S. Kaplan of the Department of Radiology.

liters of the same buffer used for electrophoresis. All electrophoresis experiments were conducted under the following conditions: buffer, 0.02 molar phosphate containing 0.1 molar sodium chloride, pH 7.95, bath temperature, 3° C., and a potential gradient of 5 to 6 volts cm^{-1}. Each electrophoresis was continued until complete separation of the protein components had occurred. The magnified projections of the diagrams obtained were traced and the areas under the peaks measured by means of a planimeter. Areas were calculated relative to one another in terms of per cent. Absolute amounts of each fraction in grams could then be calculated from the total protein content determined on the pooled serum samples.

Mice were immunized by daily intraperitoneal injection of 0.5 ml. of a 2 per cent suspension of washed sheep erythrocytes. In one group of 20 mice, antigen administration was stopped when the blood agglutinin titer was 1 to 700, and in a second group of 12 animals when the titer reached 1 to 2560. The mice were then allowed to remain in the laboratory without further antigen administration. In order to test the anamnestic response to X-rays, the first group of mice received radiation when no titer was present in the blood, and the second group when the serum agglutinin titer was 1 to 80. Serum obtained from the heart blood of each mouse was used and agglutinin titers were done by the doubling dilution method.

RESULTS

Adrenal cholesterol. Injection of purified adrenotrophic hormone in normal mice is followed within a few hours by a depletion of the sudanophilic material of the adrenal cortex and a marked decrease in the total cholesterol content of the adrenals (Dougherty and White, 1944). These alterations in adrenal composition have been interpreted as evidence of secretion of adrenal cortical steroid hormones. Various stimuli which augment pituitary-adrenal cortical secretion cause similar changes in adrenal lipids (Sayers, Sayers, Fry, White and Long, 1944). Figure 1 shows data establishing that X-radiation is another stimulus which produces increased secretion of pituitary adrenotrophic hormone. The adrenals of normal mice given 200 r show within a few hours a marked decrease in total cholesterol content. The decrease in adrenal cholesterol persists for as long as 48 hours after irradiation. Disappearance of sudanophilic material parallels the cholesterol changes in these mice. It has also been demonstrated that as small a dose as 10 r will decrease the sudanophilic material of the adrenal cortex of normal mice within 3 hours following radiation.

Effect on blood lymphocyte levels. Irradiation of normal mice with 10 r or 200 r, and normal rats with 200 r, produces a decrease in the absolute number of circulating lymphocytes (Figs. 2 and 3). In normal mice given 10 r, the lymphopenic effect is maximal at 3 to 6 hours after exposure to X-rays. Although the lymphopenia is not as pronounced with this dose as that seen with higher doses in mice, e.g., 200 r, statistical analysis of the data has shown that the decrease in numbers of circulating lymphocytes is significant. In normal mice or

rats exposed to 200 r, the maximal lymphopenic effect is observed at a later time, i.e., 24 hours after irradiation, is more profound, and persists for longer periods of time. The adrenalectomized mice given 10 r, on the other hand, do not show a significant lymphopenia during the 48-hour period of study. The larger dose of radiation, 200 r, produces a profound, absolute lymphopenia in adrenalectomized mice

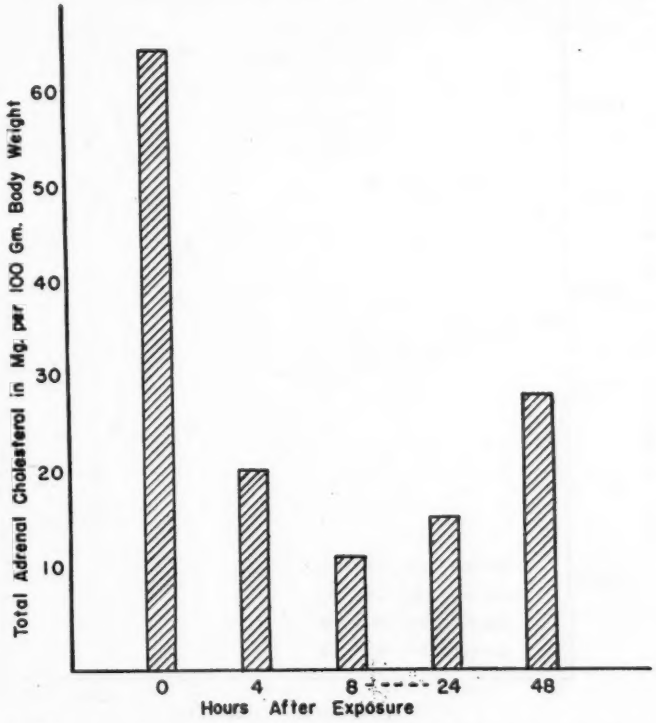


FIG. 1. Effect of a single exposure to 200 r on the total adrenal cholesterol of normal mice. Each column represents the average of values obtained on three individual animals.

or rats, and in hypophysectomized rats. The effect in the operated animals with the larger radiation dose is similar to that seen in normal animals given the same degree of exposure.

Normal mouse blood contains chiefly two types of leucocytes, the lymphocytes, which comprise approximately 80 per cent of the total white cells, and the heterophiles which account for the remainder of the leucocytes. Therefore, an increase in total white cell count accompanying an absolute lymphopenia must be due to an increased number of circulating heterophiles. This is seen to a striking degree in normal and adrenalectomized mice receiving 200 r (Fig. 4). Although

the data are not presented, a marked stimulation of heterophile production was also seen in adrenalectomized and hypophysectomized rats exposed to 200 r.

The lymphopenias which were observed following radiation could

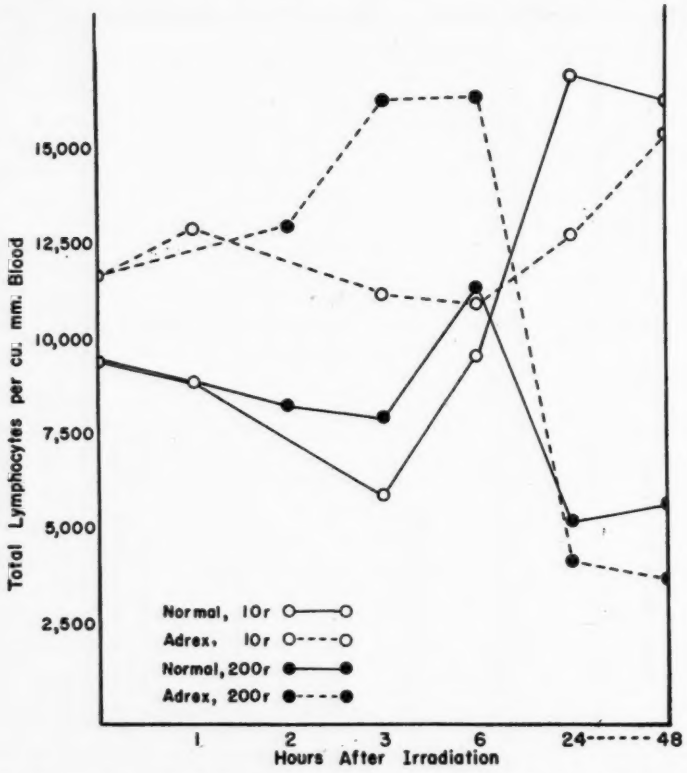


FIG. 2. Effect of a single dose of 10 r or 200 r on the total lymphocyte count of normal and adrenalectomized mice. The control values for normal mice represent data for 99 animals: the same values for the adrenalectomized mice were obtained on a group of 60 animals. Each of the other points on the chart is an average of the individual counts for a group of 5 mice.

not be due to hemodilution inasmuch as a slight increase in whole blood specific gravity was observed in the experimental animals. Also, the lymphopenia occurring in mice given the low Roentgen dose was not due to the handling of the mice prior to irradiation. This point was specifically investigated in view of a private communication from Dr. L. Jacobsen of the University of Chicago to the effect that in his laboratory, male mice of the ABC strain showed as significant a lymphopenia following handling as that seen after a single exposure to

10 r. In the present experiments, however, mice placed in the box used for treatment, but not subjected to irradiation, showed no decrease in blood lymphocyte levels over an 8-hour period. This observation of Jacobsen further indicates the extreme sensitivity of the blood lymphocyte levels to stress. The recent investigation of Elmadjian

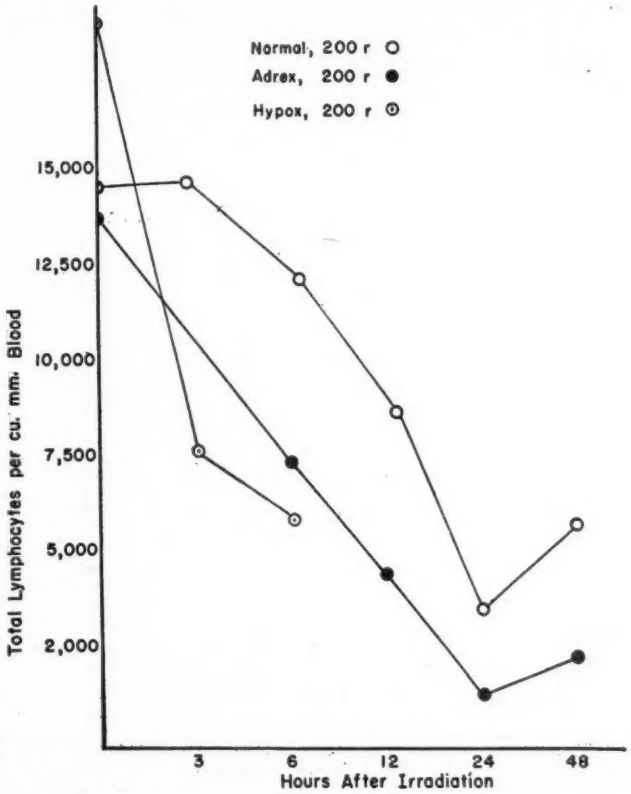


FIG. 3. Effect of a single dose of 200 r on the total lymphocyte count of normal, adrenalectomized and hypophysectomized rats. Each point on the chart is an average of the individual counts obtained for a group of from 3 to 6 rats.

and Pincus (1945) has emphasized this point in relation to the handling of mice.

Structural alterations in lymphoid tissue. The histological alterations produced in the lymphoid structures of normal mice subjected to X-radiation were essentially those described by other investigators (Murphy, 1926; Latta and Ehlers, 1931; Selling and Osgood, 1938; Henshaw, 1944a, b). In general, the changes consisted in a degeneration of lymphocytes characterized by loss of staining capacity and

karyorrhexis (Fig. 6). Following the initial dissolution of lymphocytes (Fig. 6), there was a development of macrophages which ingested the nuclear debris resulting from the necrosis of lymphocytes (Fig. 8). The lymph nodes became edematous and there was a generalized swelling of reticuloendothelial cells. The disappearance of lympho-

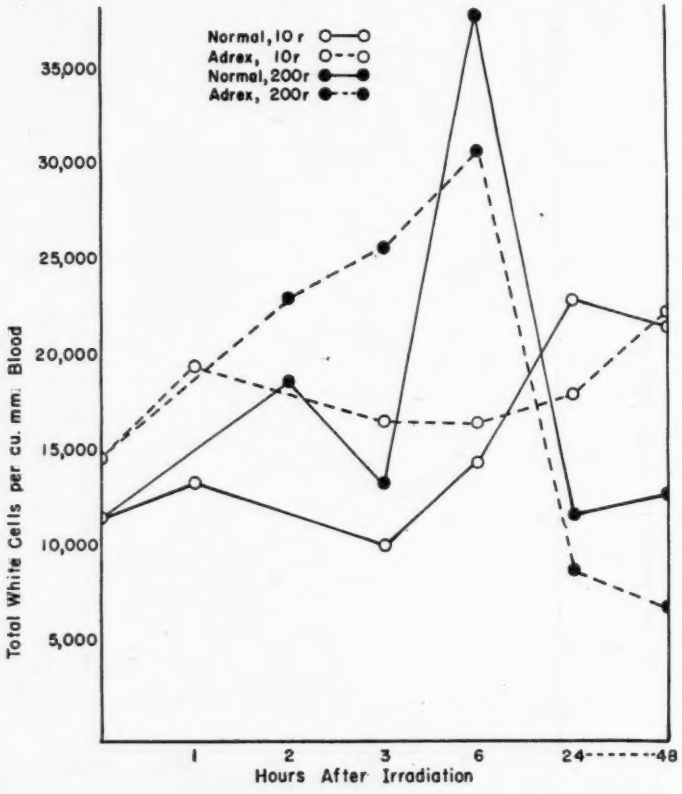


FIG. 4. Effect of a single dose of 10 r or 200 r on the total white cell count of normal and adrenalectomized mice. Each point on the chart represents the same number of animals as in figure 2.

cytes from the lymphoid tissues resulted in a decreased size of these organs. Essentially similar changes occurred in all of the lymph nodes studied (axillary, inguinal, mesenteric and cervical), the thymus (Fig. 8) and the Peyer's patches. Although the white pulp of the spleen was most affected, lymphocytes in the red pulp also showed marked damage. In general, the changes were similar to, but not identical with, those seen following injection of adrenotrophic or adrenal cortical hormones (Dougherty and White, 1945).

The above-described alterations are seen in varying degrees in normal mice given either 10 r or 200 r, and in adrenalectomized mice given 200 r (Figs. 6, 7, 8, 11, 12). However, adrenalectomized mice exposed to 10 r showed only very slight changes in lymphocyte structure (Figs. 9, 10).

The most extensive dissolution of lymphocytes occurred 3 to 5 hours following radiation with 10 r, although slight changes were observed as early as 1 hour after exposure. The lymphoid tissues of adrenalectomized mice radiated with a 10 r dose showed only a very

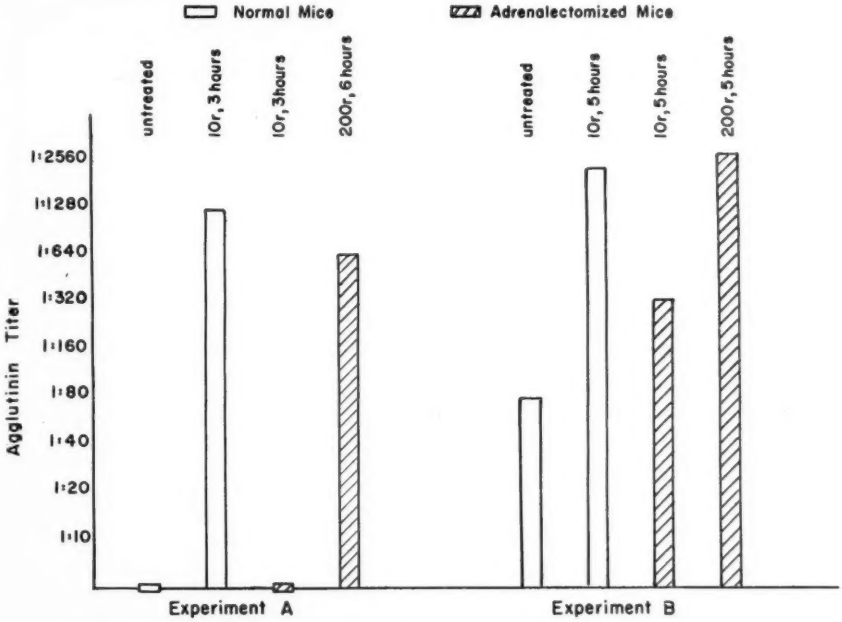
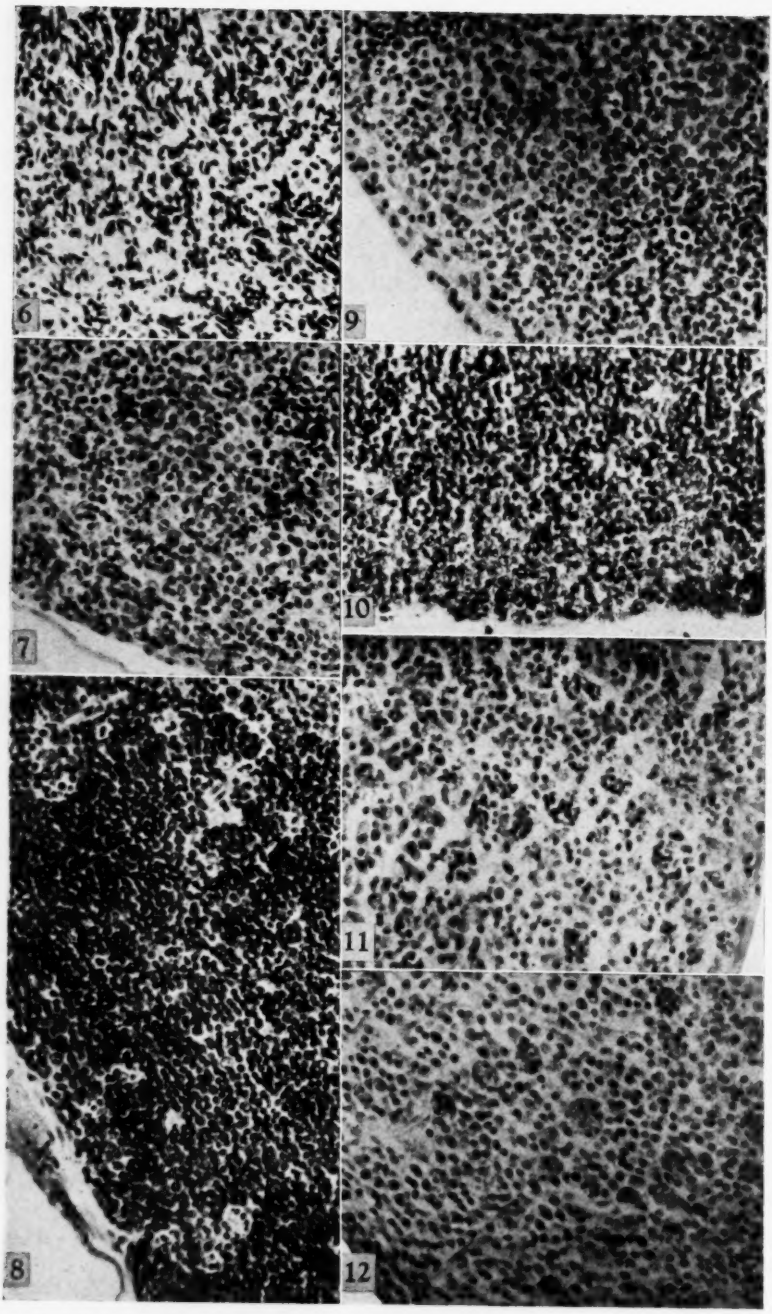


FIG. 5. Effect of a single exposure to X-rays on the anamnestic response. In experiment A, each column represents the average value obtained from individual titrations in a group of 5 mice. In experiment B, the values represented by each column were obtained in a group of 3 mice.

mild lymphocytic change which was confined primarily to the peripheral lymph nodes. In a group of 10 adrenalectomized mice examined from 3 to 5 hours after exposure to 10 r, only one animal's lymphoid structures showed alterations comparable to those seen in the unoperated mice given the same radiation dose.

In the normal mice given the high radiation dose, 200 r, maximum changes in lymphocytes were seen within 4 to 8 hours following exposure and persisted to some degree for at least 24 hours. Animals given the higher radiation dose had significantly smaller lymphoid structures, as compared to untreated mice, for as long as 48 to 72 hours



FIGS. 6–12. See opposite page for description.

following treatment. The adrenalectomized mice exposed to 200 r exhibited an essentially similar tissue picture as that described for the unoperated animals given the same radiation dose. Here again the maximum alterations were obtained in 4 to 8 hours after treatment. It is of interest that the thymus of these animals was more affected than were the other lymphoid tissues. The most significant histological difference between the normal and adrenalectomized mice given 200 r was the extremely rapid reconstitution of lymphoid tissue which occurred in the operated animals. Within 24 hours following exposure, the lymphoid tissue of the adrenalectomized mice had not only regained its normal size, but had attained the typically hyperplastic appearance characteristic of the adrenalectomized animal (Grollman, 1936; Reinhardt, 1943; Dougherty and White, 1945).

More detailed observations regarding changes in lymphoid tissue other than those described here will be reported at a later time.

FIG. 6. Mesenteric lymph node of normal mouse 4 hours following whole body radiation with 10 r. The pycnosis of lymphocytes and disappearance of these cells from the meshes of the stroma were more marked in the lymphatic structures of this animal than was usually observed with this dosage of X-rays. Sectioned at 5 micra; H. and E. stain. ×325.

FIG. 7. Inguinal lymph node of normal mouse 2 hours following whole body radiation with 10 r. Marked karyorrhexis of lymphocytes and phagocytosis of nuclear debris in germinal center. The formation of reaction centers following radiation is the most distinctive change in the lymphatic tissues of normal animals receiving the lower dosages of X-rays. Sectioned at 5 micra; H. and E. stain. ×325.

FIG. 8. Thymus of normal mouse 4 hours following whole body radiation with 10 r. The pitted appearance of the thymus is due to the large numbers of macrophages ingesting nuclear debris. This change is one of the most characteristic findings in the thymus of normal animals receiving lower dosages of X-rays. Sectioned at 5 micra; H. and E. stain. ×325.

FIG. 9. Lymph node of adrenalectomized mouse 4 hours following whole body radiation with 10 r. Reaction centers are not found in lymphatic tissues of adrenalectomized animals exposed to low dosage of X-rays. Although a few hyperchromatic nuclei are observed here and there in the lymph node, they are not phagocytized nor are they observed in any restricted areas. Such cells are also seen in lymph nodes of non-treated adrenalectomized mice. Sectioned at 5 micra; H. and E. stain. ×325.

FIG. 10. Thymus of adrenalectomized mouse 4 hours following whole body radiation with 10 r. There is no pitting of the thymus of adrenalectomized animals following exposure to low dosage of X-rays. The thymus of adrenalectomized animals is so cellular that it is difficult to get an even section. The small dark cells are probably indicative of direct X-ray effect at this dosage although such hyperchromatic small lymphocytes are observed in the thymus of normal, adrenalectomized animals. Sectioned at 5 micra; H. and E. stain. ×325.

FIG. 11. Mesenteric lymph node of adrenalectomized mouse 8 hours following whole body radiation with 200 r. The depletion of small lymphocytes from the meshes of the stroma, lack of normal staining capacity and the extensive karyorrhexis of lymphocytes are typical of the X-ray effects on lymphatic tissues. At this stage there was no significant difference between normal or adrenalectomized animals receiving this dosage of X-rays. Sectioned at 5 micra; H. and E. stain. X325.

FIG. 12. Thymus of adrenalectomized mouse 8 hours following whole body radiation with 200 r. The alterations induced by this high dosage are similar to those shown in the previous figure. No significant difference was observed between the thymus of normal or adrenalectomized animals at 8 hours following exposure to 200 r. Sectioned at 5 micra; H. and E. stain. ×325.

Alterations in serum proteins. The data obtained from electrophoretic studies of the sera of normal mice and mice exposed to X-rays are presented in table 1. Irradiation of normal mice with 10 r did not produce a marked change in total protein, albumin or alpha globulin concentration of the blood. There was a slight rise in the beta globulin concentration and a large increase in the amount of gamma globulin. In the adrenalectomized mice exposed to 10 r, there was a slight increase in the percentages of all of the globulin fractions. However, the amount of globulin in grams increased slightly only in the gamma globulin fraction. Treatment of either normal or adrenalectomized mice with 200 r produced a striking elevation in the gamma globulin fraction of the serum, with lesser rises evident in the beta globulin portion. The alterations in beta globulin

TABLE 1. SERUM PROTEIN ALTERATIONS IN MICE FOLLOWING IRRADIATION WITH X-RAYS*

Type of animal	Radiation dose	Total protein	Albumin		α-Globulin		β-Globulin		γ-Globulin	
	r	gm./%	%	gm.	%	gm.	%	gm.	%	gm.
Normal, control	—	6.02	74.9	4.5	8.6	0.52	7.9	0.47	8.6	0.53
Normal	10	5.89	66.8	3.9	7.8	0.46	9.8	0.57	15.5	0.92
Adrenalectomized	10	5.08	66.6	3.4	11.2	0.57	9.4	0.46	13.0	0.66
Normal	200	6.55	69.0	4.5	5.9	0.39	8.8	0.57	16.4	1.07
Adrenalectomized	200	6.41	50.9	3.3	15.1	0.97	11.9	0.76	22.3	1.43

* Mice exposed to 10 r were bled 3 hours after irradiation; animals given 200γ were bled 6 hours following X-ray treatment.

recall those seen following injection of adrenotrophic hormone (White and Dougherty, 1945 c) and support the suggestion (White and Dougherty, 1945c, 1946) that beta globulin may also be a normal constituent of lymphocytes. The alpha globulin of adrenalectomized, but not of normal, mice also increased following exposure of the animals to 200 r.

Induction of anamnestic response. The enhancement of antibody titer which occurs following the injection of a variety of substances other than the original antigen has been called the anamnestic response or reaction. This response is manifested as an increase in antibody in the blood of previously immunized animals. Induction of the anamnestic response was studied in two groups of animals. In one group, the immunized mice remained in the laboratory without further antigen administration and until no circulating antibody was demonstrable (3 to 6 months). In the other group of mice, the anamnestic response was elicited when the blood titer had dropped to a low, but measurable level. The data are shown in figure 5. Exposure of normal mice to 10 r produced a marked anamnestic response. On the other hand, adrenalectomized mice, with no circulating antibody,

showed no agglutinin titer 3 hours after exposure to 10 r. It may be pointed out that the sera of these animals showed a slight (1 to 20) hemolysin titer. In the other group of mice with initial low blood titers, 10 r again produced a marked increase in the agglutinin titer of the blood, whereas adrenalectomized mice given 10 r showed only a slight rise in serum agglutinins.

In contrast to the above data, a large radiation dose of 200 r produced a marked anamnestic response in the absence of the adrenal glands.

DISCUSSION

The number of circulating lymphocytes is dependent on at least two major factors: the rate of delivery of lymphocytes from the sites of their synthesis, and the rate of peripheral removal of these cells. These factors have been carefully evaluated by Drinker and Yoffey (1941). One of the important regulators of the rate of delivery of lymphocytes to the circulation is pituitary-adrenal cortical secretion (Dougherty and White, 1944, 1945; Reinhardt and Li, 1945). Moreover, it has been demonstrated that only those adrenal cortical steroids oxygenated in position 11 of the steroid nucleus exert this controlling influence (Dougherty and White, 1945). The lymphopenic effect of adrenal cortical steroids is a reflection of lymphocyte dissolution in lymphoid organs (Dougherty and White, 1945). Conversely, the lymphocytosis of adrenalectomized animals (White and Dougherty, 1945b) is probably due to the absence of the hormonal mechanism normally controlling lymphocyte dissolution.

The lymphopenic effect of Roentgen radiation has been demonstrated in numerous clinical and experimental studies (Russ, 1921; Minot and Spurling, 1924; Murphy, 1926; Mayneord and Piney, 1928; Selling and Osgood, 1938; Dunlap, 1942). Although investigators are generally agreed that large doses of radiation produce a profound and prolonged lymphopenia, Murphy (1926) has demonstrated that smaller degrees of radiation may result in a lymphocytosis within 24 hours after exposure. However, Mottram and Russ (1921) observed that the initial effect of low doses of X-rays was a lymphopenic one, followed within 24 hours by a lymphocytosis. The data in the present paper support the conclusion of Mottram and Russ (1921) and others (Dunlap, 1942) that a lymphopenia is seen with widely varying dosages of radiation.

In other experiments not presented here, it has been found that adrenalectomized animals given a larger radiation dose (100 r) showed an equally profound destruction of tissue lymphocytes but no lymphopenia was evident. This is in contrast to the early, profound lymphopenia seen following injection of adrenotrophic hormone (Dougherty and White, 1944). It is interesting to note that although the lymphopenia seen with hormone is initiated prior to extensive damage to tissue lymphocytes, the reverse situation holds with

respect to the relation of blood and tissue changes following radiation. It is possible that the hormonal mechanism may have accelerated peripheral removal of lymphocytes.

Histological examination of lymphoid structures of radiated animals revealed that dissolution of lymphocytes was probably the basis for the observed lymphopenia. The tissue alterations seen with the smaller doses of radiation in normal mice were not as extensive as those resulting from large degrees of radiation. Although histological changes occurred in several adrenalectomized animals exposed to 10 r, they were restricted to a few peripheral organs. The lymphoid tissues of normal or adrenalectomized mice exposed to larger dosages (50 to 200 r) of radiation showed the same degree of extensive lymphocytolytic effects.

The alterations in lymphoid tissue histology seen following exposure of normal mice to X-rays are similar in type to, but much more extensive in nature than, those observed (Dougherty and White, 1945) shortly after the injection of either adrenotrophic hormone or adrenal cortical extract. The alterations in histological structure occur earlier and persist longer following large doses of radiation than after hormone injection.

The data which have been presented suggest that X-rays, unlike a wide variety of unrelated physical and chemical stimuli which cause lymphoid tissue dissolution, do not depend on adrenal cortical mediation for their lymphocytolytic action. The lymphopenia seen in hypophysectomized, irradiated rats would suggest that pituitary mediation, whether via the adrenal cortex or other endocrine glands, is not required for the lymphoid tissue involution produced by X-rays. It is recognized that other agents may exist, which if administered in suitable concentration might also produce lymphocyte dissolution in the absence of the pituitary or the adrenals.

The extensive changes in the lymphoid tissues of subjects irradiated in a localized area (Leblond and Segal, 1942; Osgood, 1942) has raised the question of whether the action of X-radiation on lymphoid tissue is due to a direct action or to a humoral influence. Radiation of tissue cultures (Osgood, 1942; Stenstrom, King and Henschel, 1942) and of suspensions (Schrek, 1946) of lymphocytes indicates that lymphocytes are affected by as small a dose as 20 r. On the other hand, other investigators (reviewed by Dunlap, 1942) reported that lymphocytes *in vitro* exhibited a high degree of radioresistance. Osgood (1942), has suggested that lymphocytes injured locally in the area of exposure are carried by the circulation into various lymphoid structures where they undergo lysis. Another explanation of the generalized lymphocytolysis induced by X-rays can be postulated on the basis of the results obtained in the present investigation. It is suggested that X-rays have both a direct and an indirect effect on lymphocytes. Thus, the lymphoid tissue directly ex-

posed to X-radiation undergoes involution. This is seen in the normal and adrenalectomized mice exposed to a total body radiation of 50 to 200 r. An indirect action of X-rays on lymphoid tissue can be explained on the basis of the increased pituitary-adrenal cortical activity caused by radiation.

Striking support for the hypothesis that the indirect effects of X-rays may be dependent on a humoral mechanism is seen in the recent work of Barnes and Furth (1943) who have shown that radiation of one of a pair of parabiotic mice, the other of which is shielded, caused extensive lymphoid tissue changes and lymphopenia in both animals. Furthermore, Leblond and Segal (1942) observed that whereas exposure of only the posterior one-third of normal rats to X-rays caused generalized lymphoid tissue involution, very large radiation doses given to adrenalectomized animals did not produce alteration in lymphoid structures distant from the exposed area. Leblond and Segal (1942) also found that there was a direct effect of X-rays on lymphocytes in that radiation of the thymic area produced atrophy of this organ in the adrenalectomized animal, although other lymphoid structures were unaffected.

The normal dissolution of lymphocytes which occurs continually in lymphoid structures results in the release of serum globulin to the circulation. One of the proteins of lymphocytes is identical with normal serum gamma globulin (Kass, 1945; White and Dougherty, 1945c, 1946) and the rate of release of this protein from lymphocytes is under pituitary-adrenal cortical control. Consequently, any stimulus which augments pituitary-adrenal cortical secretion may produce an increase in the concentration of the globulin fraction of the serum proteins. Large doses of radiation, producing an extensive degeneration of lymphocytes in normal or adrenalectomized animals, therefore also produce an increase in serum globulins. This response in adrenalectomized animals supports the working hypothesis that dissolution of lymphocytes is the mechanism by which these cells release protein to the circulation. The serum protein changes again indicate that the smaller degree of radiation (10 r) is acting as a non-specific stimulant and producing its major effects on lymphoid tissue structure and function by way of the pituitary-adrenal cortical mechanism.

The increases in gamma globulin seen following radiation have been confirmed by a study of the effect of X-rays on the anamnestic response. The anamnestic response is a reflection of the pituitary-adrenal cortical control of the release of antibody globulin from lymphocytes. (Dougherty, Chase and White, 1945; White and Dougherty, 1946). The ability of X-rays to release immune globulin from lymphocytes is of interest in relation to the reported capacity of radiation to increase resistance to infections (Desjardins, 1931, 1937, 1942; Bisgard, Hunt, Neely and Scott, 1942; Dunlap, 1942). Although the anamnestic response has been employed to emphasize the

pituitary-adrenal cortical control of the rate of *release* of immune globulin from lymphocytes, it is recognized that during an anamnestic response *synthesis* of new globulin may also have occurred.

The data for the anamnestic response reveal that there is an increase in circulating antibody shortly after exposure of a previously immunized animal to a single dose of X-rays. The results are not in conflict with those of Hektoen (1915, 1918) and of Murphy and Sturm (1925) who demonstrated that chronic doses of X-rays, sufficient to maintain lymphoid tissue involution, decreased the capacity of the organism to form antibodies. This is not surprising since the conditions employed by these investigators produced a marked decrease in the number of those cells now known to be concerned with antibody formation.

SUMMARY

CBA mice, 60 to 80 days old, received total body X-radiation. A large dose of X-rays, 200 r, produced within 3 hours a lymphopenia, tissue lymphocyte degeneration, and total serum protein and gamma globulin increases. These changes also occurred in 1 day postoperative adrenalectomized mice receiving 200 r. This dose gave an anamnestic response in previously immunized mice in the absence of the adrenals.

It has been demonstrated that 10 r produced the same physiological alterations, including the anamnestic response, in normal, but not in adrenalectomized, mice. Therefore, 10 r influenced lymphoid tissue function by augmenting pituitary-adrenal cortical secretion. Large doses of X-radiation may produce lymphocyte degeneration without adrenal mediation and thus increase circulating gamma globulin and antibodies. This is further proof that adrenal cortical steroids produce hyperglobulinemia by their degenerative effects on lymphocytes, an end cell of adrenal cortical hormone action.

It is suggested that X-rays exert both a direct and an indirect effect on lymphocytes. The direct action may be manifested even in the absence of the pituitary or adrenals, whereas the indirect action of X-radiation on lymphocytes is mediated via the pituitary-adrenal cortical mechanism.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the advice and aid given by Dr. H. S. Kaplan of the Department of Radiology during the course of this investigation. Miss Ruth Shrader contributed valuable technical assistance.

REFERENCES

BARNES, W. A., AND O. B. FURTH: *Am. J. Roentgenol.* 49: 662, 1943.
BISGARD, J. D., H. B. HUNT, O. A. NEELY AND P. SCOTT: *Radiology.* 38: 477. 1942.
DESJARDINS, A. U.: *J.A.M.A.* 96: 401. 1931.
DESJARDINS, A. U.: *Radiology* 29: 436. 1937.

DESJARDINS, A. U.: *Radiology* 38: 274. 1942.
DOUGHERTY, T. F., J. H. CHASE AND A. WHITE: *Proc. Soc. Exper. Biol. & Med.* 58: 135. 1945.
DOUGHERTY, T. F., AND A. WHITE: *Endocrinology* 35: 1. 1944.
DOUGHERTY, T. F., AND A. WHITE: *Am. J. Anat.* 77: 81. 1945.
DRINKER, C. K., AND J. M. YOFFEY: Lymphatics, lymph and lymphoid tissue. Harvard Univ. Press. Cambridge. 1941.
DUNLAP, C. E.: *Arch. Path.* 34: 562. 1942.
ELMADJIAN, F., AND G. PINCUS: *Endocrinology* 37: 47. 1945.
GROLLMAN, A.: The Adrenals. Williams and Wilkins Co. Baltimore. 1936.
HENSHAW, P. S.: *J. Nat. Cancer Inst.* 4: 477. 1944a.
HENSHAW, P. S.: *J. Nat. Cancer Inst.* 4: 503. 1944b.
HEKTOEN, L.: *J. Infect. Dis.* 17: 415. 1915.
HEKTOEN, L.: *J. Infect. Dis.* 22: 28. 1918.
KAGAN, B. M.: *J. Clin. Investigation* 17: 373. 1938.
KAGAN, B. M.: *J. Lab. & Clin. Med.* 26: 1681. 1941.
KASS, E. H.: *Science* 101: 337. 1945.
LATTA, J. S., AND O. C. EHLERS: *Am. J. Anat.* 47: 447. 1931.
LEBLOND, C. P., AND G. SEGAL: *Am. J. Roentgenol.* 47: 302. 1942.
MAYNEORD, W. V., AND A. PINEY: *Brit. J. Radiol.* 1: 257. 1928.
MINOT, G. R., AND R. G. SPURLING: *Am. J. M. Sc.* 168: 215. 1924.
MOTTRAM, J. C., AND S. RUSS: *J. Exper. Med.* 34: 371. 1921.
MURPHY, J. B.: Monographs of The Rockefeller Institute. No. 21. 1926.
MURPHY, J. B., AND E. STURM: *J. Exper. Med.* 41: 245. 1925.
OSGOOD, E. E.: *Am. J. Roentgenol.* 48: 214. 1942.
REINHARDT, W. O.: in Essays in Biology, p. 489. Univ. of Calif. Press. 1943.
REINHARDT, W. O., AND C. H. LI: *Science* 101: 360. 1945.
RUSS, S.: *Arch. Radiol. and Electrotherap.* 26: 146. 1921.
SAYERS, G., M. A. SAYERS, E. G. FRY, A. WHITE AND C. N. H. LONG: *Yale J. Biol. & Med.* 16: 361. 1944.
SCHREK, R.: *Radiology* 46: 395. 1946.
SELLING, L., AND E. E. OSGOOD: in Handbook of Hematology. Edited by H. Downey 4: 2693. 1938. Paul B. Hoeber, Inc. New York.
STENSTROM, W., J. T. KING AND A. F. HENSCHEL: *Radiology* 38: 477. 1942.
WHITE, A., AND T. F. DOUGHERTY: *Federation Proc.* 4: 109. 1945a.
WHITE, A., AND T. F. DOUGHERTY: *Endocrinology* 36: 16. 1945b.
WHITE, A., AND T. F. DOUGHERTY: *Endocrinology* 36: 207. 1945c.
WHITE, A., AND T. F. DOUGHERTY: *Ann. N. Y. Acad. Sci.*, 46: 859. 1946.

THE DISTRIBUTION OF THE FEULGEN AND 2,4-DINITROPHENYLHYDRAZINE REACTIONS IN NORMAL, CASTRATED, ADRENALECTOMIZED AND HORMONALLY TREATED RATS

S. ALBERT[1] AND C. P. LEBLOND
From the Department of Anatomy, McGill University
MONTREAL, CANADA

BENNETT (1940) introduced phenylhydrazine hydrochloride as a reagent for the histochemical study of the adrenal cortex. A yellow reaction was observed, which he attributed to the presence of ketonic lipids, presumably the keto-steroids elaborated by the gland. Later, the same reaction was found in the interstitial cells of the testis (Pollock, 1942), in the thecal and luteal cells of the ovary (Dempsey and Bassett, 1943), and in the syncytial layer of the placenta (Wislocki and Bennett, 1943), presumably indicating the presence of the specific keto-steroids produced by these organs (Dempsey and Wislocki, 1946).

It was decided to examine the phenylhydrazine reaction in tissues whose activity depends on the presence of keto-steroid hormones. This reaction was found to be present in the seminal vesicles and prostate of the male rat. Castration, on the other hand, produced a marked decrease of the staining intensity. However the reaction did not disappear completely, even when adrenalectomy was performed in addition to castration, thus removing the two known possible sources of keto-steroids in males. This result threw some doubt on the specificity of the phenylhydrazine reaction as a test for keto-steroids.

Gomori (1942) had criticized the specificity of Bennett's reaction and emphasized that the adrenals as well as sections from different kinds of necrobiotic tissues (tubercles, infarcts, tumors) reacted not only with phenylhydrazine, but also with Feulgen's leucofuchsin reagent as used in the plasmal reaction. This reaction is believed to identify in tissue sections a group of substances named "plasmalogens," which comprise acetals of fatty aldehydes (stearic, palmitic, etc.) bound with colamine glycerophosphate (Thannhauser and Schmitt, 1946). Gomori was of the opinion that Bennett's reaction was due to plasmalogens rather than to keto-steroids. Aboim (1943) also noted that the results obtained with phenylhydrazine and Feulgen reagent in the adrenal cortex were quite similar and concluded that the significance of these reactions was questionable. On the other hand, a number of authors suggested that Feulgen's plasmal reaction,

Received for publication October 1, 1946.
[1] Fellow of the Donner Foundation.

like phenylhydrazine, could be used for the histochemical detection of keto-steroids (Dempsey and Wislocki, 1944; 1946).

It was obviously important to reach a definite conclusion on this matter. It was first decided to study the comparative distribution of the Feulgen and phenylhydrazine reactions in order to see how comparable the results of these two reactions were throughout the body. In addition, this distribution was examined in male rats deprived of the two known sourses of keto-steroids, namely testes and adrenals, as well as in animals treated with testosterone or desoxycorticosterone acetate.

METHODS

1. STAINING METHODS

a) 2,4-Dinitrophenylhydrazine stain. It was possible to improve Bennett's technique and obtain a more intense color in thinner sections resulting in better cellular localization. This was accomplished by using Eastman Kodak 2,4-dinitrophenylhydrazine (DNPH)[2] instead of phenylhydrazine, and by clearing the tissues in 17 per cent alcohol. After 48 hours fixation in 10% formalin neutralized with magnesium carbonate, the tissues were washed in running water for 24 hours and sectioned at 10–15 micra on a freezing microtome. In our first experiment the sections were treated with iodine and thiosulphate with the intention of removing the ascorbic acid (Bennett, 1940). It seemed unnecessary to continue this procedure as its omission did not affect the intensity or appearance of the hydrazone; the ascorbic acid presumably dissolved out during fixation and subsequent washings. Therefore, in our more recent experiments this step was eliminated. After fixation the sections were cleared for 4 hours in 17% alcohol; this concentration of alcohol did not dissolve out the substances reacting with DNPH, as occurred with higher concentrations of alcohol. It had been noted in gross preliminary experiments that the keto-steroid testosterone was insoluble in 17% alcohol. The sections were then carried to a saturated solution of DNPH in 30% alcohol to which a sufficient amount of 0.2N sodium acetate had been added to raise the pH to neutrality; this decreased the alcohol content of the DNPH solution to about 17%. The sections were left in this solution overnight, washed in 17% alcohol for 20 minutes to remove any excess of stain, carried into distilled water and mounted in glycerine-gelatine. A positive reaction was indicated by a yellow color in the tissues.

b) Feulgen Reaction. This reaction was carried out as indicated by Lison (1936). After 48 hours fixation in 10% neutral formalin and washing in running water, the freezing microtome sections were left in sublimate of mercury for 3 minutes, carried into a solution of leucofuchsin, washed in sulphurous acid, and mounted in glycerine gelatine. A positive reaction was indicated by a purple color in the tissues. In a number of cases, the DNPH and Feulgen reactions were carried out simultaneously with and without pretreatment with sublimate.

---

[2] We wish to express our thanks to Dr. H. Sobel, who suggested the use of DNPH. Dempsey and Wislocki (1946) mentioned that this substance was used in their laboratory by Pechet.

2. EXPERIMENTAL

Two series of male albino rats were investigated.

The first series comprised 4 groups of 5 or 6 mature rats weighing 154 ± 10 grams. The animals in the first group were normal controls; in the second, castrated for 7 days; in the third, castrated for 21 days; in the fourth, castrated and given subcutaneous injections of 1 mg. of Testosterone in 0.1 cc of Mazola oil twice daily starting from the day of operation up to the seventh day, when they were sacrificed.

The second series consisted of 6 groups or 4 to 8 immature male albino rats weighing from 60 to 85 gms. The animals in the first group were normal controls; in the second, castrated for 21 days; in the third, adrenalectomized for 14 days; in the fourth, castrated for 21 days and adrenalectomized for 14 days; in the fifth, castrated for 21 days, adrenalectomized for 14 days and given subcutaneous injections of 1 mg. of testosterone suspended in 0.1 cc of water twice daily for 7 days; in the sixth, castrated for 21 days, adrenalectomized for 14 days, and given subcutaneous injections of 1 mg. of desoxycorticosterone acetate (Percorten Ciba) in 0.2 cc of oil twice daily for 7 days. All the animals were sacrificed at the same time.

The animals of the first series were kept on water and purina fox chow ad lib, and were fasted for fourteen hours before being sacrificed. The second series received purina fox chow and pablum ad lib; the animals which had been adrenalectomized were given 1% saline to drink; the other animals had access to water. The animals were sacrificed by means of chloroform. Tissues removed at autopsy were immediately placed in 10% neutral formalin.

Castration was performed by the ventral abdominal route, both testes and their epididymi being removed. The bilateral adrenalectomies were performed through the dorsal route. The suprarenal region of the adrenalectomized animals was carefully checked at autopsy to see if the operation had been complete.

3. EVALUATION OF THE RESULTS

Following autopsy all organs were fixed and stained as indicated above. The intensity of the staining method was graded in pluses on a scale of 0 to 4+. In the following description the adjective "intense" refers to 4+, "fairly intense" to 3+, "moderate" to 2+, and "slight" to 1+.

The slides were not counterstained and were photographed by using the same exposure time for the sections to be compared. In printing the positives a poorly staining area was picked out and brought to the same intensity in the photographs to be compared. For instance, in the adrenal, the non-staining medulla was brought to the same intensity in all comparable groups; in the seminal vesicles the muscle was used as a guide. A Wratten purple filter was used to accentuate the yellow dinitrophenylhydrazones. Some unstained details may be apparent in the plates due to lowering of the microscope condenser.

RESULTS

1. GENERAL DISTRIBUTION IN NORMAL CONTROLS

The distribution of the purple reaction revealed by the Feulgen reagent (Imhaüser, 1927; Verne, 1929) and of the yellow dinitro-

phenylhydrazones revealed by the DNPH was very widespread extending to almost all organs and tissues. As far as we could ascertain the general distribution of the two reactions was identical throughout. Cytological details stood out more clearly in the slides stained with DNPH as cellular damage was produced by the drastic reagents of the Feulgen reaction. On the other hand the intensity of staining with the latter was much greater. At any rate, the results of both reactions being identical, they will be reported as such.

Endocrine Glands. In the endocrine glands the most intense reaction was given by the adrenal cortex (fig. 2N). The reaction varied in intensity being maximal in the fascicular and glomerular zones with usually an unstained band between the two zones. The reticular zone stained less intensely, but a definite reaction was present in all the cells, sharply demarcating them from the colorless cells of the medulla. Within the cells the reaction was present in the lipid globules.

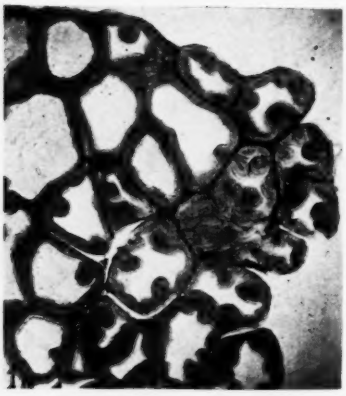
The anterior lobe of the hypophysis showed a slight reaction located in the basophilic and possibly in the acidophilic cells. The intermediary lobe did not react. The posterior lobe only showed clumps of brightly staining globules in a few scattered cells.

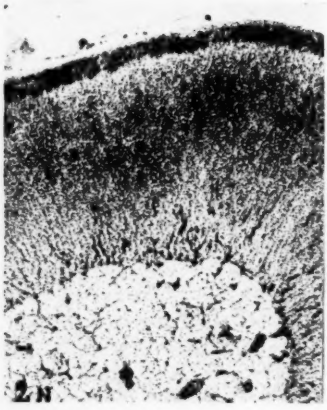
In the thyroid a moderate reaction was found in the follicular epithelium but not in the colloid (fig. 7 and 8). The parathyroid cells did not react. The islets of Langerhans showed none or a slight color.

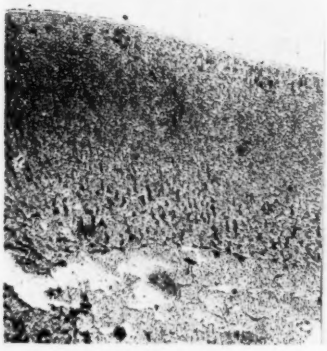
In the testis a slight reaction was found in the interstitial cells. Pollock (1942) reported a marked reaction with phenylhydrazine in the interstitial cells of cats, rats, guinea pigs, mice and rabbits. We were unable to confirm his results with the rat but obtained an intense reaction in the interstitial cells of the testis of year old C57 mice. Furthermore in the rat, single bright shining globules may occasionally be found in the periphery of the seminiferous tubules, presumably in the Sertoli cells. In addition, the tubules containing sperm nearing maturity showed reactions on round or oval structures adjacent to sperm heads; these structures appeared to be the cytoplasm eliminated from the spermia during the formation of the sperm.

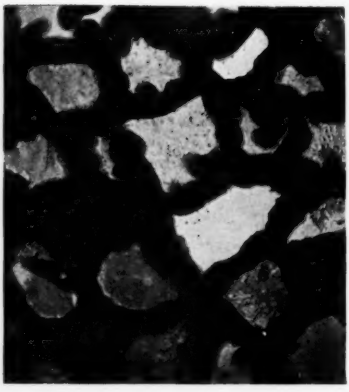
The ovaries of a few pregnant and non-pregnant female rats were also investigated and showed a reaction in the cells of the corpus luteum and in the thecal and interstitial cells. Within the cell the reaction was located in the lipid globules.

Acessory Sex Organs. In the male an intense reaction was found in the prostatic epithelium (figs. 1N and 5N), where it extended throughout the cytoplasm and appeared accentuated at the level of the supranuclear golgi apparatus. No reaction was found either in the secretion or in the interstitial tissue. In the seminal vesicles (fig. 3N and 6N) there was a fairly intense reaction located in the epithelium; this reaction was present in the vacuoles of the cell apex described by Moore, Hughes and Gallagher (1930). These so-called vacuoles are









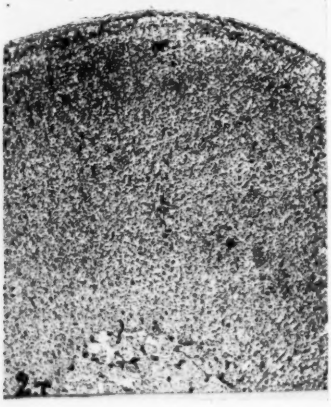


PLATE 1

formed of a light zone with a dark corpuscle in the center. The stain seemed to locate in the light zone. The basal cytoplasm showed only a slight reaction. As in the prostate the secretion present in the lumen did not stain. The epithelium of the epididymis showed a fairly intense reaction. No reaction was found in the tubular contents (sperm and secretion). The preputial glands showed an intense reaction in both cells and sebum.

In the female rat the epithelium of the mammary gland showed a slight reaction.

Other Organs. The liver showed a fairly intense reaction, which under high magnification appeared present throughout the cell. In the kidney (figs. 11 and 14) an intense reaction was given by the proximal convoluted tubules; a moderate one, by the distal convoluted tubules and Henle's loop; none, by the tubes of Bellini and the glomeruli. This pattern clearly emphasized the subdivision of the kidney into three zones: cortex intensely stained on account of the proximal convoluted tubules; outer medulla moderately stained on account of Henle loops and distal convoluted tubules, and the inner medulla colorless on account of the tubes of Bellini.

In the nervous system an intense reaction was given by myelin sheaths. This was found in the peripheral nerves (fig. 7 and 8, N) as well as in the nerve tracts of the central nervous system (fig. 9 and 10).

In the gastro-intestinal tract there was only a slight reaction or none at all. The intestinal epithelium reacted slightly. The gastric glands and the epithelium did not react appreciably. In the salivary glands the reactions were slight; except for a moderate reaction in the excretory ducts of the submaxillary gland. The pancreatic acini showed a moderate reaction. In general, mucus tissues (goblet cells of the intestine, mucus acini of the sublingual salivary gland) did not stain.

In the lungs a slight reaction was evident. However the epithelium of the trachea and bronchi showed a moderate reaction.

In the skin the layers of keratin showed a slight reaction. The hard

---

Stain—DNPH. The grey to black color represents the yellow seen in the tissue sections. The black spots are usually due to blood in the blood vessels.

FIG. 1N. Normal rat prostate. Epithelium succulent and staining intensely. Secretion does not stain.

FIG. 1C. 21 day castrate rat prostate. Epithelium atrophic and staining slightly.

FIG. 1T. Testosterone treated, 7 day castrate rat prostate. Epithelium succulent and staining very intensely. Secretion does not stain.

FIG. 2N. Normal rat adrenal. Glomerular zone staining intensely; fascicular, moderately, with a colorless band between the two; reticular, slightly and medullar not at all.

FIG. 2C. 21 day castrate rat adrenal. Diminution in staining intensity of the glomerular and fascicular zones. No change in the reticular and medullar zones.

FIG. 2T. Testosterone treated, 7 day castrate rat adrenal. Same as in Fig. 2C.

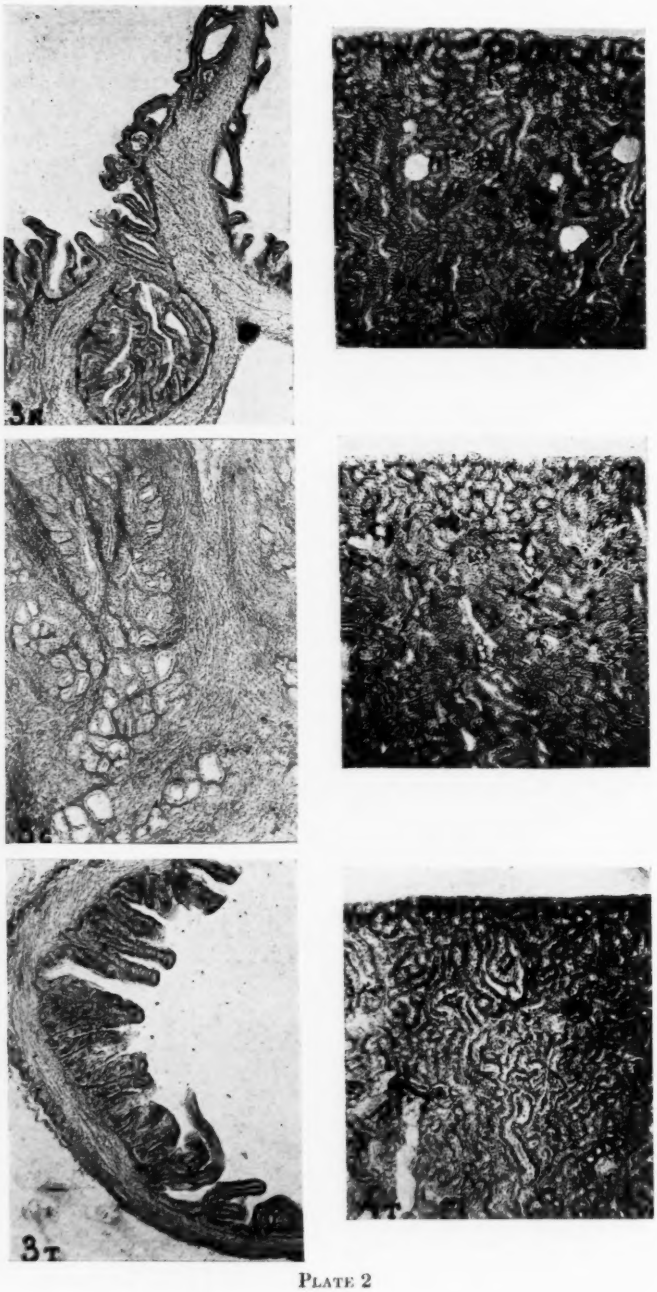


PLATE 2

keratin of the hair did not react. A slight or moderate reaction was found in the sebaceous glands.

In the spleen, the red pulp was doubtful. The lymphatic nodules were also doubtful as far as lymphocytes and lymphoblasts were concerned. But some cells in the reticulum were laden with clumps of brightly staining globules. Similarly, in the thymus, no reactions were encountered except for an intense one in some of the reticular cells. It may be noted that identical cells with irregular, deeply staining globules were also found in the posterior hypophysis. All these cells should be considered as part of the reticulo-endothelial system.

Connective tissue cells and collagenous fibers did not react. But a fairly intense reaction was observed in the elastic tissue; this could be seen in the elastic fibers scattered throughout the connective tissue, as well as in the internal elastic membrane of arterial walls (fig. 12 and 13). Fat cells (fig. 7 and 8, F) showed all types of reaction from intense to none at all; intensely staining globules may be seen close to non-reacting ones. In cartilage cells sudanophilic globules may be found which reacted with both reagents.

The reaction of muscle tissue varied according to the type of muscle. The smooth muscle fibers stained only slightly or not at all; the striated muscle fibers of the skeleton stained moderately (fig. 7 and 8,M) and cardiac fibers, fairly intensely.

Finally it must be emphasized that in all organs and tissues the nuclei never reacted with either the Feulgen or DNPH stain.

2. GENERAL DISTRIBUTION FOLLOWING BIOLOGICAL INTERVENTIONS

From the first series of experiments (Table 1) it appeared that the prostate and seminal vesicles of castrated rats lost to a considerable extent and some even completely their ability to react with either DNPH (fig. 1C and 3C) or Feulgen's reagent (fig. 5C and 6C). At seven days after castration the reaction was moderate and at twenty-one days, slight or absent. In the case of the seminal vesicles it is known that the apical vacuoles of Moore disappear after castration. The two reactions decreased gradually as these vacuoles disappeared. In addition, rare, scattered, small globules of sudanophilic material

---

Stain—DNPH. The grey to black color represents the yellow seen in the tissue sections. The black spots are usually due to blood in the bood vessels.

FIG. 3N. Normal rat seminal vesicle. Epithelium succulent and staining fairly intensely. Concentration of the color in the apical portion of the cells.

FIG. 3C. 21 day castrate rat seminal vesicle. Epithelium atrophic and staining slightly. Muscle as in Fig. 3N.

FIG. 3T. Testosterone treated, 7 day castrate rat seminal vesicle. Epithelium succulent and staining moderately. Color concentrated in the apical portion of the cell.

FIG. 4N. Normal rat kidney cortex. Intense reaction in the proximal convoluted tubules.

FIG. 4C. 21 day castrate rat kidney cortex. No change from Fig. 4N.

FIG. 4T. Testosterone treated, 7 day castrate rat kidney cortex. No change from Figs. 4N and 4C.

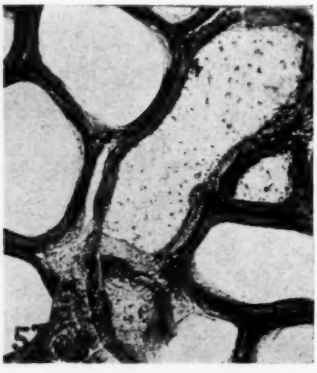


PLATE 3

may appear in the cells of the castrates; these globules stained intensely with both reagents.

Injection of testosterone restored the reaction in prostate and seminal vesicles of castrated animals (Table 1). In the prostate, this restoration was well marked, since the reaction after the hormone treatment may be more intense than in normal controls (fig. 1T and 5T). In the seminal vesicles, on the other hand, the reaction was somewhat less intense in the treated castrates than in normal controls (fig. 3T and 6T). On the whole, it may be stated that testosterone

TABLE 1. EFFECT OF VARIOUS INTERVENTIONS IN MATURE MALE RATS ON THE STAINING OF MALE ACCESSORIES WITH DNPH AND FEULGEN REAGENT

Group	No. of animals	Initial body weight (gm.)	Final body weight (gm.)	Seminal vesicles weight (mg.)	Intensity of Reaction		
					Seminal vesicles	Ventral prostate	Preputial gland
Normal Controls	6	154 (154–160)	213 (200–225)	480 (320–605)	+++	++++	++++
7-day Castrates	6	155 (145–160)	159 (150–165)	85 (67–138)	++	+	++++
21-day Castrates non-injected	5	154 (145–160)	203 (190–215)	47 (35–56)	±	±	++++
7-day Castrates, Testosterone	5	155 (145–160)	164 (150–175)	791 (595–943)	++	++++	++++

restored a staining ability within the range of the normal animal. The reaction in the preputial glands was not appreciably affected by either castration or treatment with testosterone.

None of the other organs examined was influenced by either castration or testosterone injections in so far as color intensity and distribution were concerned (fig. 4N, C, T). The only exception to this statement was the adrenal cortex. Changes in the color intensity of the adrenal cortex with phenylhydrazine-hydrochloride have already been described in shocked animals by Popjak (1944) and in pantothenic deficient rats by Deane and McKiblin (1946). In the present

Stain—Feulgen's plasmal reagent. The grey to black color represents the purple of the tissue sections.

FIG. 5N. Normal rat prostate. Epithelium succulent and staining fairly intensely. Secretion unstained.

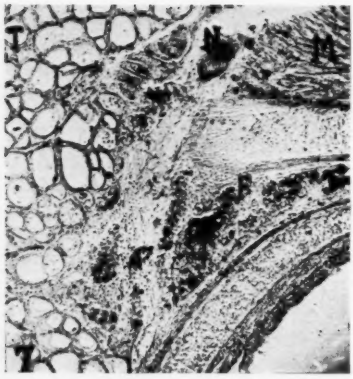
FIG. 5C. 21 day castrate rat prostate. Epithelium atrophic and staining slightly.

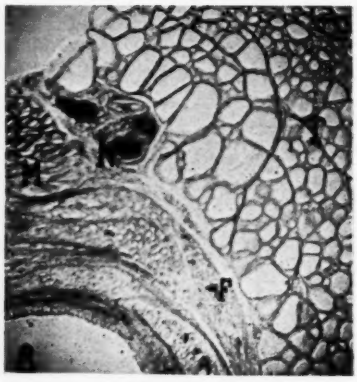
FIG. 5T. Testosterone treated, adrenalectomized, castrate rat prostate. Epithelium succulent and staining intensely. Secretion unstained.

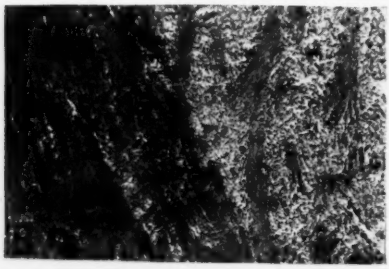
FIG. 6N. Normal rat seminal vesicle. Epithelium succulent and staining fairly intensely. Concentration of the color in the apical portion of the cells.

FIG. 6C. 21 day castrate rat seminal vesicle. Epithelium atrophic and staining slightly. Muscle as in Fig. 6N.

FIG. 6T. Testosterone treated, adrenalectomized, castrate rat seminal vesicle. Epithelium succulent and staining moderately. The color concentrated in the apical portion of the cell. Muscle as in Figs. 6N and NC.







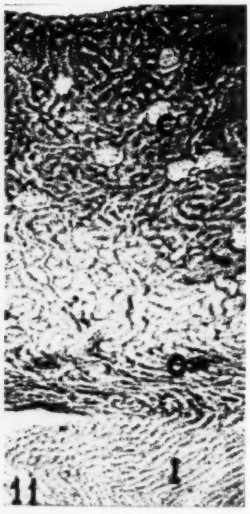


PLATE 4

series it was found that castration produced a decrease in the color intensity in the glomerular and fascicular zones (fig. 2C). Examination under high power showed that the globules stained in the normal adrenal cortex were reduced in size in the castrates. Testosterone at the present dose level did not appear to restore the normal picture in the adrenal cortex (fig. 2T). It must be remembered that in this series all animals had been fasted for fourteen hours prior to being

TABLE 2. EFFECT OF VARIOUS INTERVENTIONS IN IMMATURE MALE RATS ON THE STAINING OF MALE ACCESSORIES WITH DNPH AND FEULGEN REAGENT

Group	No. of animals	Initial body weight (gm.)	Final body weight (gm.)	Seminal vesicles weight (mg.)	Ventral prostate weight (mg.)	Preputial gland weight (mg.)	Intensity of reaction		
							Seminal vesicles	Ventral prostate	Preputial gland
Normal Controls	4	70 (60–85)	150 (135–170)	134.5 (64–197)	118 (118–128)	62.5 (38–87)	++	+++	++
21 day Castrates	4	70 (60–80)	130 (100–150)	7 (5–9)	9.5 (5–14)	45 (29–41)	+	+	+++
14 day Adrenalectomized	3	70 (60–80)	113 (110–120)	69 (45–95)	77.5 (62–94)	36 (22–52)	++	+++	+++
21 day Castrates +14 day Adrenalectomized	4	68 (60–80)	130 (120–150)	5 (4–6)	12.5 (8–16)	23 (16–34)	+	+	+++
21 day Castrates +14 day Adrenalectomized +Testosterone	3	69 (60–85)	123 (100–145)	126 (104–155)	106 (96–119)	53 (52–64)	+	+++	++
21 day Castrates +14 day Adrenalectomized+Desoxycorticosterone	6	72 (60–85)	128 (115–140)	8.5 (5–15)	9.5 (6–11)	28.5 (24–36)	+	0	+++

sacrificed and this may have played a part in the changes of the staining intensity of these glands.

In the second series of experiments, performed on immature male rats (Table 2), it was also found that castration markedly decreased and testosterone restored the reactions observed in the prostate and seminal vesicles. However, the staining intensity of the seminal vesicles in normal controls was less than in the more mature animals of the first series. The decrease in the staining intensity of the adrenals following castration was not clearcut in this series.

As far as the reactions in all other organs were concerned, it was not possible to detect any consistent action of castration or testosterone treatment.

Adrenalectomy and desoxycorticosterone acetate treatment caused

Stain—Feulgen's Reagent—Figs. 7, 9, 11, 12.

DNPH—Figs. 8, 10, 13, 14.

The grey to black color represents either the purple of the Feulgen Reaction or the yellow of the DNPH as seen in the tissue sections.

FIGS. 7 and 8. Normal thyroid (T), Nerve (N), Muscle (M), and Fat (F).

FIGS. 9 and 10. Normal brain. The myelin of the nerve tracts staining intensely. The grey matter, slightly.

FIG. 11 and 14. Normal kidney showing the intensely staining cortex (C) with the non-staining glomeruli and the poorly staining outer (O) and inner (I) medulla.

FIG. 12 and 13. Arteriole showing the darkly staining internal elastic membrane.

no variation from the controls in the staining of the tissues with the two reagents.

It must be emphasized that in both series none of the hormonal interventions affected the general pattern of distribution and intensity of the reaction, except in the seminal vesicles, prostate and adrenals. Furthermore both stains ran parallel throughout all organs and tissue.

DISCUSSION

From the point of view of the histochemist, the main conclusion of these experiments was the parallelism between the results obtained with DNPH and the Feulgen reagent in normal and variously treated animals. Two explanations seemed possible; first, the two reactions were due to 2 types of substances closely associated in the body, one being plasmalogen and the other a distinct aldehyde or ketone. However, Feulgen, Imhäuser and Behrens (1929) and Verne (1940) showed that if a tissue section was treated with phenylhydrazine, the plasmal reaction could no longer be obtained. This indicated that the substance responsible for the plasmal reaction was also able to react with phenylhydrazine.

This led to the second possibility, namely that a single type of substance was responsible for the two reactions. The keto-steroids might be considered in this regard, because the reactions were present in cellular structures which were known to elaborate these hormones (adrenal cortex, corpus luteum, interstitial cells in the testes). Furthermore, since steroids have been reported to occur in the seminal vesicles of the rat (Delor, 1942), our finding of DNPH and Feulgen reactions in this organ appeared to support the opinion that these reactions were due to steroids. This point of view was further strengthened by the observation that castration decreased and testosterone restored the staining intensity of the seminal vesicles and prostate (Table 1). However, these tissues becoming atrophic after castration might not only lose any steroids present but also a number of other biologically active components. Therefore, these experiments did not necessarily imply a role of the steroid hormones in the above reactions.

On the other hand, in animals castrated for 21 days there remained usually a slight reaction in the vesicles and prostate. It was thought at first that this slight reaction might be due to the presence of a small amount of keto-steroids of adrenal cortical origin. It was therefore decided to carry out a second series of experiments in which the animals would be deprived of both testes and adrenals (Table 2). Here again a slight reaction was found in the seminal vesicles and prostate suggesting that a substance other than a testicular or adrenal cortical hormone was involved. Furthermore, the removal of both the adrenals and testes did not affect the reactions present throughout all other organs and tissues of the body. This again eliminated the role of a keto-steroid originating from either testis or adrenal. Any remaining doubt was removed when experiments in vitro with testo-

sterone, progesterone and brei of kidney and liver were performed, in order to evaluate the reactivity of these substances with Feulgen's reagent according to the method of Feulgen and Gruenberg (1939). In contrast with the plasmalogens which were shown by Feulgen to produce an intense purple color in minute doses, testosterone and progesterone in doses of 30 mg. were only slightly reactive. By comparing the color intensities obtained with the tissue brei and the keto-steroids it could be calculated that progesterone or testosterone would have to be in concentrations of 1 in 10 or 1 in 2 respectively in the tissues in order to account for the color intensity developed by the tissue brei. It is obviously impossible to find such concentrations of steroids in tissues; for instance, Allen and Goetsch (1936) found that the concentration of progesterone in sow's ovaries was of the order of magnitude of 1 part in 40,000. It seemed necessary, therefore, to look for other substances that might be responsible for both the Feulgen and DNPH reactions.

There is good evidence that most, if not all plasmalogens—or substances producing Feulgen's plasmal reaction—are loosely bound aldehydes of high fatty acids. The strict parallelism between the Feulgen and the DNPH reaction suggested that plasmalogens were also responsible for producing the DNPH reaction. The only objection to this interpretation was that the Feulgen reaction is classically believed to occur only after treatment of the tissues with sublimate of mercury. Indeed it was our experience that sublimate treatment considerably intensified the reaction; but in the absence of treatment with sublimate, the reaction was still present although weaker. Aboim reported similar results (1943). Extensive experiments were therefore made in which the intensity of the staining with DNPH was compared in sections with and without pretreatment with sublimate. These experiments showed that sublimate markedly increased the intensity of staining with DNPH. In fact the effect of sublimate was quite comparable in both the Feulgen and DNPH reactions, as it intensified the color and blurred the cytological details of the two reactions.

It could therefore be concluded that the histochemical reactions obtained with phenylhydrazine and its derivatives were due to the agents effective in producing the Feulgen reactions, namely "plasmalogens." Plasmalogens were found to be present in many organs and tissues, especially in normally functioning seminal vesicles and prostate, where they were maintained by the action of either testosterone injections or physiological androgens.

SUMMARY

There is a strict parallelism between the histochemical reactions produced by 2,4-Dinitrophenylhydrazine and Feulgen's plasmal reagent in rat tissues. The substances producing these two reactions are very widespread throughout the body. They are present in a number of lipid rich tissues, such as adrenal cortex, corpus luteum, mammary

gland, preputial glands, myelin sheaths of the nerves, and fat cells themselves. These substances are also found in a number of organs with little or no sudanophilic components, such as muscles, kidney, liver, thyroid, and, in the male, seminal vesicles and prostate. Doubtful reactions were encountered in lung, thymus, spleen, parathyroids, etc.

Castration, in addition to producing the well recognized atrophy of seminal vesicles and prostate, causes a considerable decrease in the reaction of these organs; and testosterone restores it to an approximately normal level. Neither castration, nor testosterone affect the intensity or distribution of the reaction in any other organ or tissue (with the exception of the adrenal). Adrenalectomy, in addition to castration, does not modify the reaction anywhere.

Two types of substances may be considered as causing these reactions: keto-steroids and plasmalogens. The persistence of the reactions after ablation of the two known sources of keto-steroids in the male rat, namely testes and adrenals, runs counter to the first hypothesis; so does inability of progesterone and testosterone to markedly react with Feulgen reagent in vitro. On the other hand, the increase of both reactions with mercuric bichloride suggests that "plasmalogens" are the effective agents.

ACKNOWLEDGEMENTS

This work was supported by grants from the Blanche Hutchison Fund of McGill University and from the Donner Foundation, Cancer Research Division. We are greatly indebted to Dr. E. Lozinski of Charles E. Frosst and Company for supplying the testosterone and progesterone used in these experiments, and to Dr. H. Racicot of Ciba Company Limited for supplying the desoxycorticosterone acetate (percorten).

REFERENCES

ABOIM, A. N.: *Bull. Soc. Port. des Sc. Natur.* 14: 119. 1943.
ALLEN, W. M., AND G. GOETSCH: *J. Biol. Chem.* 116: 653. 1936.
BENNETT, H. S.: *Am. J. Anat.* 67: 151. 1940.
DEANE, H. W., AND J. M. MCKIBLIN: *Endocrinology* 38: 385. 1946.
DELOR, J.: *Soc. Biol.* 136: 787. 1942.
DEMPSEY, E. W., AND D. L. BASSETT: *Endocrinology* 33: 384. 1943.
DEMPSEY, E. W., AND G. B. WISLOCKI: *Endocrinology* 35: 409. 1944.
DEMPSEY, E. W., AND G. B. WISLOCKI: *Physiol. Rev.* 26: 1. 1946.
FEULGEN, R., AND H. GRUENBERG: *Ztschr. f. physiol. Chem.* 257: 161. 1939.
FEULGEN, R., K. IMHÄUSER AND M. BEHRENS: *Ztschr. f. physiol. Chem.* 180: 161. 1929.
GOMORI, G.: *Proc. Soc. Exper. Biol. & Med.* 51: 133. 1943.
IMHÄUSER, K.: *Biochem. Ztschr.* 186: 360. 1927.
LISON, L.: *Histochimie animale.* Gauthier-Villars, Paris. 1936.
MOORE, C. R., W. HUGHES AND T. F. GALLAGHER: *Am. J. Anat.* 45: 109. 1930.
POLLOCK, W. F.: *Anat. Rec.* 84: 23. 1942.
POPJAK, G.: *J. Path. & Bact.* 56: 485. 1944.
THANNHAUSER, S. J., AND G. SCHMIDT: *Physiol. Rev.* 26: 275. 1946.
VERNE, J.: *Ann. de Physiol.* 5: 245. 1929.
WISLOCKI, G. B., AND H. S. BENNETT: *Am. J. Anat.* 73: 335. 1943.

EFFECTS OF HYPOPHYSECTOMY, CASTRATION, AND TESTOSTERONE PROPIONATE ON HEMOPOIESIS IN THE ADULT MALE RAT[1]

ROGER C. CRAFTS[2]

From the Department of Anatomy, Boston University, School of Medicine

BOSTON, MASSACHUSETTS

VARIOUS ENDOCRINE glands and endocrine products have been found to have an influence on the process of hemopoiesis.

Hypophysectomy induces a profound anemia in the dog (Aschner, 1912), in the rabbit (Houssay, Royer, and Orias, 1931), and in the rat (Stewart, Greep, and Meyer, 1935; Meyer, Stewart, Thewlis, and Rusch, 1937; Vollmer, Gordon, Levenstein, and Charipper, 1939; Crafts, 1941). In addition, many clinical reports support the theory that the pituitary gland is concerned in some manner as a regulator of erythropoiesis.

Castration has been reported as a cause of anemia in the male rabbit (Ferrari, 1930), the male rat (Steinglass, Gordon, and Charipper, 1941), in fowl (Arvy, 1942–43), in the hampster (Stein and Jacobsen, 1944), and in the human (McCullagh and Jones, 1942).

Androgen injections induced an increased production of erythrocytes in the male rat (Steinglass, Gordon and Charipper, 1941; Vollmer and Gordon, 1941; Vollmer, Gordon, and Charipper, 1942; Finkelstein, Gordon, and Charipper, 1944), in fowl (Arvy, 1942–43; Domm, Taber, and Davis, 1943; Taber, Davis, and Domm, 1942–43), in hamsters (Stein and Carrier, 1945), and in the human male (McCullagh and Jones, 1942).

Vollmer and Gordon (1941) treated 4 hypophysectomized female rats and 1 hypophysectomized male rat with daily injections of 1.0 mg. of testosterone propionate after anemia had become established. The total erythrocyte count returned to normal levels but the hemoglobin response was not as marked. The bone marrow was reported as stimulated but no differential counts were made. This experiment was repeated on 3 hypophysectomized male rats with 2.0 mg. of testosterone propionate per day (Vollmer, Gordon, and Charipper, 1942). Similar results were obtained.

Recently (Crafts, 1946), it was shown that the anemia that is induced by hypophysectomy in the adult female rat is of the microcytic

Received for publication October 8, 1946.

[1] This study was done with the aid of a grant from the Committee on Research in Endocrinology of the National Research Council.

[2] With the technical assistance of Miss Betsy Nadell and Mr. Salvatore Lunetta.

hypochromic type, and is accompanied by a hypoplastic bone marrow in which there is a marked decrease in the number of erythroid elements. In the light of this work it seemed important (1) to determine whether hypophysectomy in the adult male rat would produce a microcytic hypochromic anemia, (2) to determine whether loss of androgen as a result of hypophysectomy could be the cause of the anemia which is induced by the removal of the pituitary gland, (3) to repeat work on castration and androgen therapy and continue it to a detailed study of the erythrocytes, and (4) to make differential counts on the bone marrow following these treatments.

METHODS

Adult male rats of three to four months of age and of the Long-Evans strain were employed in this study. Hypophysectomy was performed from the parapharyngeal approach under ether anesthesia. The completeness of the operation was checked by examination of serial sections of the pituitary capsule. Castration was performed under ether anesthesia.

Erythrocyte and white cell counts were made with standard U. S. certified pipettes and the improved Neubauer counting chamber. All counts were made by the same person. Hemoglobin determinations were made with a Hellige hemometer so standardized that 14.5 gm. equaled 100%. Blood smears were made by the two-cover-glass technique. All smears were stained with Wright's stain for 6 minutes, followed by buffer solution[3] plus Wright's stain for 5 minutes. The smears were then washed with tap water. All observations were made on regions of the smears where erythrocytes were not in contact with one another. The degree of hypochromia was determined by observation of blood smears. The diameter of the erythrocytes was determined by measuring 200 cells with an ocular micrometer.

Reticulocyte counts were made on 1000 erythrocytes and expressed as a percentage. They were stained in the following manner. A drop of a 1% solution of cresyl blue was allowed to dry on one end of a clean slide. A drop of blood was placed on the dried stain and mixed for 1 minute. The smear was then made and rapidly dried. They were counterstained with Wright's stain in the manner described above.

Blood for this work was obtained by heart puncture while the rats were under light ether anesthesia. Blood was taken from the rat within one minute of the time the animal was removed from the ether jar. Light ether anesthesia has been shown to have no effect upon the blood picture of the adult rat when heart blood is used (Crafts, 1944). At each puncture 0.1 cc. of blood was removed and placed in a small vial containing a minute amount of powdered heparin. Blood samples can be obtained in this manner many times from the same rat with no signs of injury.

The rats were dissected under chloroform anesthesia. The bone marrow was treated in the following manner. The femurs of both sides were removed and cleaned of all muscles. Each femur was then split along its whole length and one half of the bone removed. This left the bone marrow intact in a "boat" of bone which was placed in Helly's fixative. After the bone marrow

[3] 6.63 gm. monobasic potassium phosphate + 3.20 gm. dibasic sodium phosphate + 1000 cc. distilled water.

had become hard in the alcohols, it was removed from the bone in one long piece. After embedding in paraffin and sectioning at 12 micra, they were stained with toluidin blue and eosin. Differential counts were made by examining 500 cells. The degree of hypoplasia or hyperplasia was determined by observing the ratio of fat to cellular material.

The rats were maintained on the Rockland Farms complete rat diet plus fresh lettuce once a week.

PROCEDURE AND RESULTS

Controls

Table 1 shows the normal blood figures for adult male rats. These figures were obtained by a study of 274 separate blood samples from 121 rats.

TABLE 1. NORMAL BLOOD FIGURES FOR ADULT MALE RATS OBTAINED FROM A STUDY OF 274 SEPARATE BLOOD SAMPLES FROM 121 RATS

Total erythrocyte count per cu. mm.	8,920,000 ±540,000
Hemoglobin level in gm. per 100 cc.	17.6 ±0.97
Reticulocyte count in per cent	2.2 ±1.0
Total white cell count per cu. mm.	7,900 ±2,460
Neutrophils in per cent	21.5 ±6.3
Eosinophils in per cent	2.9 ±1.7
Lymphocytes in per cent	73.8 ±6.5
Monocytes in per cent	2.0 ±1.4

± =Standard Deviation.

These experiments were carefully controlled. Two or three blood samples were taken from all rats in this study before treatment was started. In addition, 14 normal control rats were studied at 10-day intervals for 50 days. The results obtained are shown in figure 1 and table 2, and are essentially similar to the results shown in table 1. The total erythrocyte and hemoglobin figures remained at normal levels.

Study of the blood smears of this group showed that occasionally a normal rat would exhibit erythrocytes with pale centers, typical of other species of animals. The majority of the animals had erythrocytes which appeared solid, however. Figures 2, 4, and 6 are examples. Erythrocytes of the control rats measured 6.4 micra (S.D., 0.03)[4] in diameter.

A slight neutrophilia and lymphopenia developed in these control rats. Table 2 shows that these changes in white cell percentages occurred in all four groups of animals. No explanation is offered for these

[4] Standard deviation.

TABLE 2. THE EFFECTS OF NO TREATMENT, HYPOPHYSECTOMY, HYPOPHYSECTOMY PLUS DAILY INJECTIONS OF 2 MG. OF TESTOSTERONE PROPIONATE, AND CASTRATION UPON THE BLOOD PICTURE OF ADULT MALE RATS

TRC = Total red cell count in millions per cu. mm.; HB = Hemoglobin in gm. per 100 cc.; Retic = Reticulocyte count in per cent; TWC = Total white cell count per cu. mm.; Neut = Neutrophils in per cent; Eosin = Eosinophils in per cent; Lym = Lymphocytes in per cent; Mono = Monocytes in per cent; BW = Body Weight; ± = Standard Deviation.

Controls—14 rats									
Day	TRC	HB	Retic	TWC	Neut	Eosin	Lym	Mono	BW
0	9.14 ±0.37	17.7 ±0.5	2.9 ±1.3	8,282 ±1,300	19.6 ±7.1	3.5 ±1.4	73.7 ±6.9	2.0 ±0.9	278 ±18.4
10	9.03 ±0.63	16.6 ±1.9	1.9 ±0.6	8,246 ±1,290	23.3 ±7.7	3.8 ±3.1	71.0 ±7.2	2.9 ±2.8	298 ±24.0
20	9.01 ±0.72	17.8 ±0.9	1.9 ±0.6	8,118 ±1,188	28.4 ±8.6	3.2 ±1.7	65.9 ±9.0	3.3 ±1.8	300 ±38.1
30	9.07 ±0.55	17.3 ±1.0	1.6 ±0.3	7,520 ±1,510	23.5 ±7.9	3.9 ±2.9	69.6 ±9.5	2.1 ±1.3	322 ±24.2
40	9.28 ±0.41	17.8 ±1.4	1.9 ±1.0	8,860 ±2,040	27.0 ±4.2	4.8 ±3.6	66.6 ±6.8	1.4 ±1.0	328 ±24.0
50	8.77 ±0.63	16.7 ±1.4	1.9 ±1.0	7,521 ±1,320	28.5 ±3.7	2.1 ±1.3	68.4 ±3.9	1.1 ±0.7	341 ±19.4

Hypophysectomized—7 rats									
Day	TRC	HB	Retic	TWC	Neut	Eosin	Lym	Mono	BW
0	9.02 ±0.31	18.0 ±0.6	3.4 ±0.4	6,879 ±2,000	18.9 ±2.8	2.4 ±0.8	77.1 ±2.8	1.6 ±0.5	266 ±31.8
10	8.61 ±0.69	16.7 ±1.3	1.5 ±0.3	9,394 ±2,800	19.2 ±7.1	3.3 ±2.1	75.6 ±6.3	1.9 ±1.0	234 ±17.7
20	6.52 ±1.87	15.8 ±1.7	2.0 ±0.4	11,233 ±6,320	22.1 ±6.2	3.9 ±0.6	71.8 ±5.7	2.0 ±2.1	220 ±20.0
30	7.52 ±0.57	14.3 ±0.7	1.7 ±0.5	12,233 ±1,610	21.8 ±2.2	2.8 ±2.2	73.2 ±2.1	2.3 ±1.5	216 ±19.1
40	5.62 ±0.44	10.1 ±0.6	2.7 ±0.7	12,275 ±2,340	23.5 ±9.4	4.8 ±2.8	68.6 ±9.4	3.1 ±0.7	195 ±15.1

Hypophysectomized plus 2.0 mg. of testosterone propionate per day—5 rats									
Day	TRC	HB	Retic	TWC	Neut	Eosin	Lym	Mono	BW
0	9.12 ±0.37	17.9 ±0.5	3.6 ±1.1	7,767 ±1,230	20.3 ±4.7	2.7 ±1.3	75.3 ±4.7	1.9 ±1.2	271 ±9.0
10	9.69 ±0.32	18.0 ±0.9	2.1 ±0.8	10,370 ±3,320	25.5 ±4.7	2.8 ±2.5	69.1 ±5.0	2.6 ±1.3	241 ±11.4
20	8.47 ±0.46	16.0 ±0.8	2.0 ±0.6	8,700 ±1,550	25.0 ±8.2	2.1 ±1.7	71.9 ±6.5	1.0 ±0.4	223 ±10.8
30	9.15 ±0.20	15.8 ±0.8	2.6 ±0.3	9,067 ±1,020	32.1 ±8.8	1.7 ±1.2	65.9 ±7.3	0.8 ±0.4	213 ±5.2
40	8.58 ±0.55	14.8 ±1.0	3.1 ±1.2	6,650 ±3,020	28.2 ±5.3	3.5 ±2.1	67.3 ±4.1	1.0 ±0.7	205 ±16.7

TABLE 2—*Continued*

Day	TRC	HB	Retic	TWC	Neut	Eosin	Lym	Mono	BW
				Castrated—12 rats					
0	8.79 ±0.47	17.2 ±0.7	2.8 ±0.9	7,189 ±1,600	20.4 ±4.8	2.7 ±1.0	75.0 ±4.5	1.9 ±1.2	280 ±28.3
10	8.49 ±0.46	16.9 ±1.4	2.2 ±1.3	8,188 ±2,750	21.6 ±6.2	4.6 ±1.7	71.5 ±6.8	2.5 ±1.6	286 ±32.2
20	8.84 ±1.26	15.9 ±1.3	3.0 ±2.7	7,350 ±1,980	22.4 ±7.5	3.4 ±2.4	72.2 ±6.5	1.3 ±1.1	274 ±17.7
30	8.49 ±1.17	16.8 ±1.1	1.5 ±0.3	8,346 ±1,790	21.3 ±5.8	3.8 ±2.8	73.8 ±6.8	1.3 ±0.9	294 ±38.1
40	8.82 ±0.59	16.9 ±1.2	2.2 ±0.6	7,369 ±1,140	20.2 ±6.3	5.0 ±4.2	73.9 ±8.0	2.0 ±2.0	280 ±9.6
50	8.96 ±0.82	16.9 ±1.2	2.3 ±1.0	9,113 ±2,750	28.9 ±13.8	4.3 ±1.9	65.5 ±10.5	1.6 ±1.3	284 ±13.0

changes. All other factors studied remained normal throughout the 50-day period.

Differential counts were made on the bone marrow. Results are

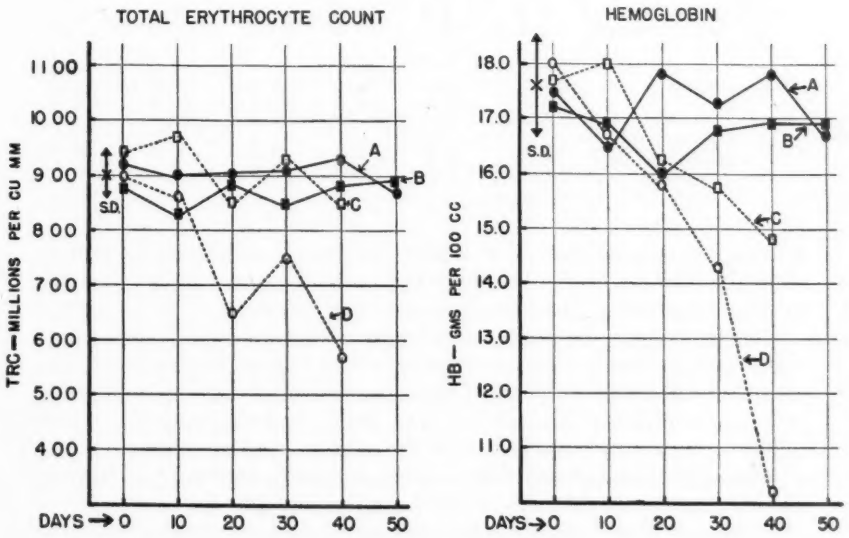


FIG. 1. The total erythrocyte counts and hemoglobin values of:
A—Normal control rats.
B—Castrated rats.
C—Hypophysectomized rats treated with daily injections of 2.0 mg. of testosterone propionate.
D—Hypophysectomized rats.
S.D. = Standard Deviation (See Table 1).

shown in table 3. The erythroid elements constituted 58.1% (S.D., 5.5) of marrow cells. Figure 8 shows a bone marrow typical of this group of normal controls. The bone marrow of normal adult male rats is more hyperplastic and contains more erythroid elements than found in normal adult female rats (Crafts, 1946).

Hypophysectomy

The finding that anemia induced by hypophysectomy in adult female rats was of the microcytic hypochromic type, accompanied by

TABLE 3. DIFFERENTIAL COUNTS ON THE BONE MARROWS OF ADULT MALE RATS. CELLS RARELY FOUND HAVE BEEN OMITTED FROM THE TABLE. ALL FIGURES ARE IN PER CENT. ± = STANDARD DEVIATION

Treatment	Hemocytoblasts	Promyelocytes	Neutrophilic elements	Eosinophilic elements	Megacariocytes	Total erythroid elements
Controls	3.8 ±0.8	6.4 ±2.4	23.5 ±3.1	7.6 ±4.6	0.2 ±0.1	58.1 ±5.5
Hypophysectomized	5.1 ±1.6	9.7 ±5.7	29.5 ±8.5	10.4 ±4.2	0.3 ±0.2	43.9 ±14.0
Hypophysectomized plus 2.0 mg. of testosterone propionate per day	4.5 ±1.3	8.9 ±2.0	27.6 ±7.8	6.2 ±2.4	0.2 ±0.2	52.6 ±9.1
Castrated	3.1 ±0.7	5.0 ±3.0	22.4 ±4.6	7.2 ±3.1	0.4 ±0.2	61.6 ±7.8
Castrated plus 2.0 mg. of testosterone propionate per day	3.4 ±1.7	4.9 ±1.3	22.8 ±3.9	4.5 ±1.2	0.4 ±0.3	63.6 ±3.0

a hypoplastic bone marrow in which there was a decrease in erythroid elements (Crafts, 1946), simplifies the problem of why such an anemia develops following removal of the pituitary gland.

To determine whether the hypophysectomized male rat would show such an anemia, 7 adult male rats were hypophysectomized. The results obtained are shown in figure 1 and table 2. These rats started, before hypophysectomy, with a mean total erythrocyte count of 9.02 million cells per cu. mm. and in 40 days dropped to an average of 5.62 million cells. Hemoglobin values decreased from 18.0 gm. per 100 cc. to 14.3 gm. within 30 days, to 10.1 gm. on day 40.

Study of blood smears from this group of rats showed that the erythrocytes were severely microcytic and hypochromic. Erythrocytes of the hypophysectomized rats averaged 5.4 micra (S.D., 0.004) in diameter, 1.0 micron smaller than found in normal rats. Figures 2 and 3 show erythrocytes of an animal before hypophysectomy and 40 days after hypophysectomy.

Total white cell counts (table 2) gradually increased from 6,879

cells per cu. mm. before hypophysectomy to 12,275 cells 40 days after hypophysectomy. This rise in total white cell count is similar to that reported in hypophysectomized adult female rats (Crafts, 1941). The differential count showed neutrophilia and lymphopenia similar to that which occurred in normal control rats.

Reticulocyte counts remained normal.

The bone marrow was hypoplastic, as can be seen in figure 9. Differential counts (table 3) showed a total erythroid percentage of 43.9 (S.D., 14.0) in contrast to 58.1% for the normal controls. The large standard deviation of 14.0% is an indication of the variability in the degree of hypoplasia of the bone marrow following hypophysectomy. The lowest percentages obtained were 23.6 and 28.2.

In summary, hypophysectomy in adult male rats produced a marked decrease in total erythrocyte count, a marked decrease in hemoglobin level, hypochromia, microcytosis, an increase in total white cell count, hypoplasia of the bone marrow, and a decrease in the number of erythroid elements.

Hypophysectomy+Testosterone Propionate

To determine whether androgens would prevent the findings obtained by hypophysectomy listed above, 5 adult male rats were hypophysectomized and given daily injections of 2.0 mg. of testosterone propionate.[5] Injections were started the day following the removal of the hypophysis. Blood was studied at 10-day intervals. Figure 1 shows the results obtained on the total erythrocyte count and the hemoglobin level. Testosterone propionate maintained total erythrocyte counts at normal levels, but hemoglobin values decreased from a normal level of 17.9 gm. per 100 cc. to 14.8 gm. after 40 days.

The erythrocytes were hypochromic but not as severely hypochromic as following hypophysectomy with no androgen injections. The cells were very slightly microcytic, measuring 5.8 (S.D., 0.19) micra, 0.6 of a micron smaller than the normal controls, 0.4 of a micron larger than following hypophysectomy with no treatment. Figures 4 and 5 show erythrocytes of a rat typical of this group before hypophysectomy and 40 days after hypophysectomy and testosterone propionate therapy.

Total white cell counts and reticulocyte percentages remained normal. Differential counts on the white cells showed the same neutrophilia and lymphopenia found in the other groups of animals (table 2).

The hypoplasia of the bone marrow which followed hypophysectomy was prevented by the testosterone propionate injections. The bone marrow was normal in appearance. Figure 10 shows the bone marrow of an animal typical of this group. Differential counts on the bone marrow (table 3) showed that the androgen injected rats had an

[5] The testosterone propionate was very kindly furnished by Dr. Erwin Schwenk of the Schering Corporation.

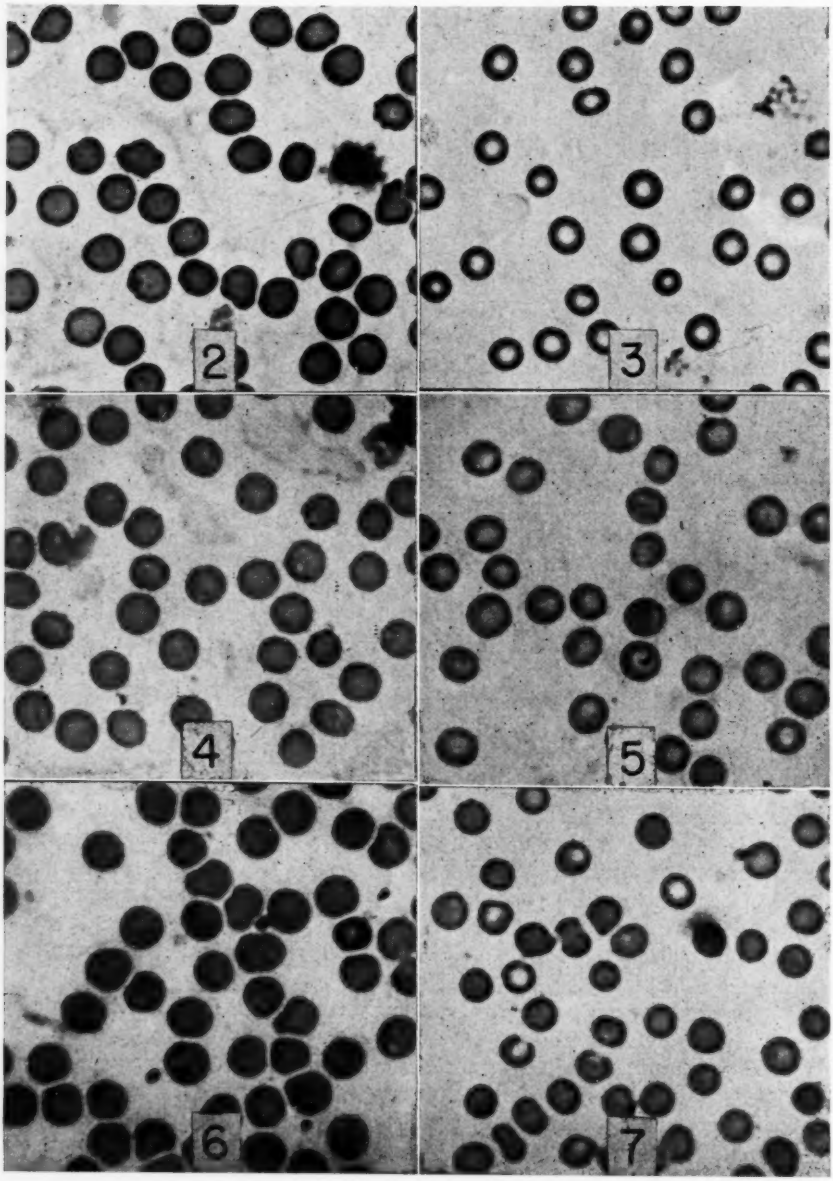


PLATE 1
Wright's stain. ×626.

erythroid percentage of 52.6, a percentage below the normal controls (58.1%), and above the hypophysectomized rats (43.9%).

The seminal vesicles and prostates were greatly enlarged.

Summarizing, daily injections of 2.0 mg. of testosterone propionate into hypophysectomized adult male rats completely prevented the drop in total erythrocyte count, the rise in total white cell count, and hypoplasia of the bone marrow; partially prevented the hypochromia, microcytosis, and the drop in erythroid elements in the bone marrow; but did not prevent the drop in hemoglobin values typical of hypophysectomized rats with no treatment. No significant changes were produced in reticulocyte percentages or differential white cell counts.

Castration

If the lack of androgen is to be considered the cause of anemia induced by hypophysectomy in the adult male rat, castration should produce a pronounced effect on hemopoiesis. Twelve adult male rats were castrated and blood studied at 10-day intervals. The results obtained on total erythrocyte counts and hemoglobin values are shown in figure 1 and further details in table 2. Although the total erythrocyte count remained at slightly lower levels throughout the 50 days the difference between these animals and the normal controls is not great. Hemoglobin values started at 17.2 gm. per 100 cc. and dropped to 15.9 gm. on day 20, and then increased to 16.9 gm. on day 50.

Fifty percent of the rats exhibited a very slight hypochromia and the other 50% no hypochromia whatever. The erythrocytes measured 5.7 (S.D., 0.29) micra in diameter, 0.7 of a micron smaller than the normal rats. Figures 6 and 7 show erythrocytes of an animal typical of this group before castration and 50 days after castration.

There were no significant changes in reticulocyte percentages, total white cell counts, or differential white cell counts (table 2).

Figure 11 shows the bone marrow of a rat typical of this group. All animals exhibited normal bone marrows in which the erythroid elements (table 3) were 61.6% (S.D., 7.8) of marrow cells.

In summary, castration in adult male rats produce a temporary drop in hemoglobin, slight decrease in total erythrocyte count, slight hypochromia in 50% of the rats studied, and microcytosis. There were no significant changes in other factors studied.

Castration+Testosterone Propionate

Five additional castrated animals were treated with 2.0 mg. of

FIG. 2. Erythrocytes of rat Gh1094 before hypophysectomy.

FIG. 3. Erythrocytes of same rat shown in figure 2, 40 days after hypophysectomy.

FIG. 4. Erythrocytes of rat B1435 before hypophysectomy.

FIG. 5. Erythrocytes of same rat shown in figure 4, hypophysectomized and treated with daily injections of 2.0 mg. of testosterone propionate for 40 days.

FIG. 6. Erythrocytes of rat Gh1184 before castration.

FIG. 7. Erythrocytes of same rat shown in figure 6, 50 days after castration.

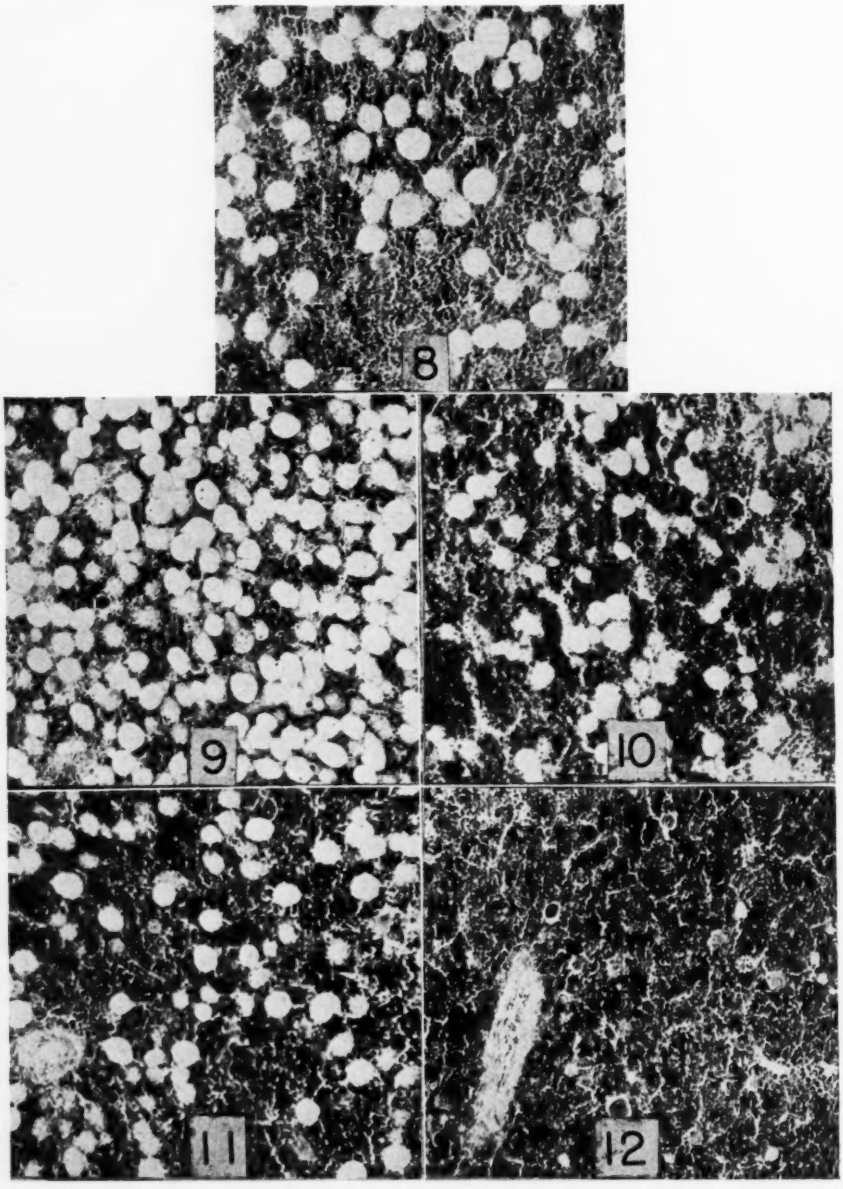


PLATE 2

Helly's fixative. Toluidin blue and eosin. 12 micra. ×87.

testosterone propionate to study the effect on the bone marrow only. Injections were started on the day following castration and continued for 50 days. All of the bone marrows were hyperplastic. Figure 12 shows the bone marrow of an animal typical of this group. The erythroid elements (table 3) were 63.6% (S.D., 3.0) of cells counted, in contrast to 58.1% for the normal controls.

These animals had greatly enlarged seminal vesicles and prostates.

DISCUSSION

That hypophysectomy produces a profound anemia in the adult rat has been reported in the literature repeatedly.[6] The data reported in this paper utilizing adult male rats support the finding in adult female rats (Crafts, 1946) that anemia induced by removal of the pituitary gland, is a microcytic hypochromic anemia which is accompanied by a hypoplasia of the bone marrow in which the number of erythroid elements is decreased.

Androgens, whether given directly or elicited by injections of gonadotropic hormone (Vollmer and Gordon, 1941), are agents which will stimulate the process of erythropoiesis. Although castration of the male rat decreased the total erythrocyte and hemoglobin levels to a level approximating that found in female rats, the decrease found was not as marked as was reported by Steinglass, Gordon, and Charipper (1941). The results obtained by the addition of androgen via testosterone propionate injections were more pronounced than those obtained by the removal of androgen via castration. Daily injections of 2.0 mg. of testosterone propionate is a large dose of androgen, however, and would seem to be more androgen per day than that produced by the testes themselves. Thus, more androgen was gained by the injections than was lost by the castration. This may be an explanation for the more pronounced effects obtained from androgen injections than from castration.

It does not seem likely that the anemia induced by hypophysectomy is due to lack of androgen. Anemia following castration does not compare in severity with that induced by hypophysectomy; removal of the hypophysis induces the same type of anemia in the

[6] For a discussion of the possible roles of water balance, loss of blood through surgery, inanition, and the removal of the posterior lobe of the hypophysis in the production of the anemia following hypophysectomy, the reader is referred to Crafts, 1941.

FIG. 8. Bone marrow of a normal adult male rat.

FIG. 9. Bone marrow of an adult male rat 40 days after hypophysectomy.

FIG. 10. Bone marrow of an adult male rat hypophysectomized and treated with daily injections of 2.0 mg. of testosterone propionate for 40 days.

FIG. 11. Bone marrow of an adult male rat 50 days after castration.

FIG. 12. Bone marrow of an adult male rat castrated and treated with daily injections of 2.0 mg. of testosterone propionate for 50 days.

female rat as is produced in male rats; and a combination of iron, copper, and thyroxine has been found to be more effective in maintaining normal erythropoiesis in the hypophysectomized rat than testosterone propionate (Crafts, 1946).

Although Vollmer, Gordon, and Charipper (1942) reported no increase in oxygen consumption in 3 hypophysectomized male rats injected with testosterone propionate, McCullagh and Jones (1942) offer very good evidence that androgens stimulate the process of erythropoiesis by increasing the basal metabolic rate. They found a correlation between the amount of androgen injected into the human and the BMR. They also found that methyl testosterone would increase the BMR to a higher level than testosterone propionate, and methyl testosterone was more effective in increasing the total erythrocyte count and hemoglobin levels.

The most effective agents for maintaining normal erythropoiesis in the hypophysectomized rat have been found to be crude pituitary extracts (Flaks, Himmel, and Zlotnik, 1937 and 1938; Querido and Overbeek, 1938), thyroxine (Meyer, Thewlis, and Rusch, 1940; Vollmer, Gordon, and Charipper, 1942; Crafts, 1946), androgens (Steinglass, Gordon, and Charipper, 1941; Vollmer and Gordon, 1941), and iron and copper plus thyroxine (Crafts, 1946). All of these substances will stimulate the general metabolism of the body, known to decrease following hypophysectomy. There is more and more evidence that the cause of the anemia which follows removal of the pituitary gland is an upset in metabolism. The metabolism of iron may be the important factor.

SUMMARY

Adult male rats of the Long-Evans strain and of 3 to 4 months of age were hypophysectomized, hypophysectomized and treated with 2.0 mg. of testosterone propionate, castrated, and castrated and treated with 2.0 mg. of testosterone propionate. Hypophysectomy caused a severe microcytic hypochromic anemia, marked decrease in hemoglobin, hypoplasia of the bone marrow, a decrease in percentage of erythroid elements in the bone marrow, and an increase in the total white cell count. Androgen therapy in hypophysectomized adult male rats prevented the decrease in the total erythrocyte count, increase in the total white cell count, and hypoplasia of the bone marrow; and partially prevented the hypochromia, microcytosis, and decrease in the total number of erythroid elements. Castration produced a slight decrease in the total erythrocyte count and hemoglobin level, a slight hypochromia, and a slight microcytosis. These changes did not compare in severity with those following hypophysectomy. Androgen therapy in castrated animals produced a hyperplasia of the bone marrow and an increase in the percentage of erythroid elements in the bone marrow. Other factors were not studied in this group. Androgens

will stimulate hemopoiesis in the rat but the decrease in androgens following hypophysectomy would not seem to be the cause of the anemia which is induced by the removal of that gland.

REFERENCES

ARVY, L.: *Le Sang.* 15: 328. 1942–43.
ARVY, L.: *Le Sang.* 15: 334. 1942–43.
ASCHNER, B.: *Pflüger's Arch. f. d. ges. Physiol.* 146: 1. 1912.
CRAFTS, R. C.: *Endocrinology* 29: 596. 1941.
CRAFTS, R. C.: *J. Lab. & Clin. Med.* 29: 1070. 1944.
CRAFTS, R. C.: *Am. J. Anat.* 79: 267. 1946.
DOMM, L. V., E. TABER AND D. E. DAVIS: *Proc. Soc. Exper. Biol. & Med.* 52: 49. 1943.
FERRARI, R.: *Haematologica.* 11: 421. 1930.
FINKELSTEIN, G., A. S. GORDON AND H. A. CHARIPPER: *Endocrinology* 35: 267. 1944.
FLAKS, J., I. HIMMEL AND A. ZLOTNIK: *La Presse Med.* 45: 1261. 1937.
FLAKS, J., I. HIMMEL AND A. ZLOTNIK: *La Presse Med.* 46: 1506. 1938.
HOUSSAY, B. A., M. ROYER AND O. ORIAS: *Rev. Soc. argent. de biol.* 7: 314. 1931.
KORENCHEVSKY, V., AND K. HALL: *J. Endocrinol.* 27: 103. 1944–45.
MCCULLAGH, E. P., AND T. R. JONES: *J.A.M.A.* 118: 1003. 1942.
MCCULLAGH, E. P., AND T. R. JONES: *J. Clin. Endocrinol.* 2: 243. 1942.
MEYER, O. O., G. E. STEWART, E. W. THEWLIS AND H. P. RUSCH: *Folia haemat.* 57: 99. 1937.
MEYER, O. O., E. W. THEWLIS AND H. P. RUSCH: *Endocrinology.* 28, 932. 1940.
QUERIDO, A., AND G. A. OVERBEEK: *Arch. internat. de pharmacodyn. et de therap.* 59: 370. 1938.
STEIN, K. F., AND E. CARRIER: *Proc. Soc. Exper. Biol. & Med.* 60: 313. 1945.
STEIN, K. F., AND B. JACOBSEN: *Anat. Rec.* 88: 459. 1944.
STEINGLASS, P., A. S. GORDON AND H. A. CHARIPPER: *Proc. Soc. Exper. Biol. & Med.* 48: 169. 1941.
STEWART, G. E., R. O. GREEP AND O. O. MEYER: *Proc. Soc. Exper. Biol. & Med.* 33: 112. 1935.
TABER, E., D. E. DAVIS AND L. V. DOMM: *Am. J. Physiol.* 138: 479. 1942–43.
VOLLMER, E. P., AND A. S. GORDON: *Endocrinology* 29: 828. 1941.
VOLLMER, E. P., A. S. GORDON AND H. A. CHARIPPER: *Endocrinology* 31: 619. 1942.
VOLLMER, E. P., A. S. GORDON, I. LEVENSTEIN AND H. A. CHARIPPER: *Endocrinology* 25: 970. 1939.

THE EFFECT OF STILBESTROL ON RESISTANCE TO EXPERIMENTAL POLIOMYELITIS

F. J. CURLEY AND W. L. AYCOCK[1]

From the Departments of Preventive Medicine and Epidemiology, Harvard Medical School and School of Public Health

BOSTON, MASSACHUSETTS

THE WIDE discrepancy between the pattern of distribution of the virus of poliomyelitis and that of the paralytic disease is an indication that added factors act as major determinants of the frank disease amongst those exposed to the virus.

Clinically the evidence for widespread dissemination of the virus lies in the unbroken gradation from frank paralytic through milder and non-paralytic forms frequently missed, to suspected abortive infections not amenable to certain diagnosis. Epidemiologically, time and space relationships between recognized cases imply intervening milder infections or healthy carriers. Immunologically, the widespread occurrence of immunity in the general population, as shown by virus neutralization tests, can best be interpreted as an expression of exposure to the virus as is true of corresponding tests in many other infectious diseases. Finally, the conception of widespread virus dissemination, originally based largely on indirect evidence, and receiving only scant laboratory verification, is now being rapidly substantiated by actual detection of virus, not only in the frank disease, but in suspected abortive infections and healthy individuals as well (Kramer, 1935), (Trask et al., 1938), (Francis et al., 1942) and (Kessel and Moore, 1945).

It is clear that many of the general features of the pattern of disease distribution are direct reflections of virus distribution. Many of the features of distribution of poliomyelitis conform to those of other infections which are distributed in accordance with well-known laws of dissemination of their infectious agents. Yet within the general pattern, selectivities in the occurrence of the paralytic disease in the small minority of those exposed to and infected with the virus serve as an indication that additional factors must enter into the determination of the outcome of the coming together of virus and host. Certain of these selectivities which can in no way be ascribed to a virus variable point to autarceologic factors which may be genetic or acquired,

Received for publication October 15, 1946.

[1] The junior author is happy to make full acknowledgment to the senior author for conceiving and executing the experiments in this paper in 1940–41 before leaving for military service. The preparation of the paper has awaited his return.

temporary or permanent, or local or general. The nature of a number of these selectivities taken together suggested experiments, the outcome of which was the enhancement of resistance of castrate monkeys to intranasal instillation of virus by the intramuscular injection of estrogenic hormone[2] (Aycock, 1940).

These experiments did not in themselves afford any indication whether the mechanism of resistance set up by administration of estrogen lay in a physiologic blockade of the nasal mucosa in the fashion of chemical blockade produced by local application (Schultz and Gebhardt, 1936), (Sabin et al., 1936), (Armstrong and Harrison, 1936) (Aycock, 1937), or in some more deep-seated structure as, for example, the CNS itself. Schultz (1941) found no increase of resistance to intranasal infection in normal monkeys following treatment of the nasal mucosa with estrogenic hormone, although changes in sexual skin indicated good response to the hormone. Kessel and Moore (1943) have reported that normal female monkeys (macaca mulatta) given 5 mg. of stilbestrol twice weekly for 1 month before inoculation and during the course of the experiment, all developed poliomyelitis as did untreated controls inoculated at the same time. In discussing these and other results in experimental poliomyelitis, they point out that the virus was inoculated intracerebrally and that differences in physiologic resistance or in pathogenesis might not occur within the central nervous system, but in extra central tissues. More recently experiments have been published in which estrogenic substance increased the resistance of the mouse to intranasal but not to intracerebral or intraperitoneal inoculation of adapted poliomyelitis virus (Foley and Aycock, 1945), and also increased resistance to oral administration of the same virus (Anderson and Bolin, 1946).

Experiments are now reported which serve to emphasize the precise set of conditions under which monkeys exhibit an increased resistance to experimental poliomyelitis under estrogenic hormone influence as previously reported (Aycock, 1940).

EXPERIMENT 1

Twenty immature normal monkeys (Macacus rhesus) were divided into four groups of two males and three females. The following preparatory treatment was then instituted. Group 1 received 0.1 mg. stilbestrol, Group 2 were given 5 mg. of testosterone propionate,[3] Group 3 received 0.1 mg. stilbestrol and at the same time 5 mg. of testosterone at a different site of injection.[4] All of the hormone injections were given intramuscularly. The fourth group were kept as un-

[2] Stilbestrol: E. R. Squibb and Son product.

[3] Oreton. Kindly supplied by Schering Corporation.

[4] The two hormones were used in this experiment to determine if there were related effects on resistance since urinary assays (unpublished data) had shown a higher excretion of both estrogen and 17-Ketosteroids in patients who had suffered poliomyelitis than in controls of corresponding age.

treated control animals. The hormone preparations were given daily for 10 days and then 3 times weekly for the duration of the experiment. Activity of the hormone preparations was indicated in all injected animals (male and female—stilbestrol and testosterone) by sexual skin changes. On the tenth day of hormone treatment all 20 animals were given intranasally 0.5 cc. of a 5 percent emulsion of monkey poliomyelitis spinal cord. The virus instillation was repeated 5 times weekly until the first monkey developed symptoms of poliomyelitis, all animals in the experiment receiving the same number of instillations. The instillations were then discontinued and the animals watched for symptoms of disease for a period of 32 days. In Group 1 (stilbestrol)

TABLE 1. EFFECT OF STILBESTROL AND TESTOSTERONE ON RESISTANCE TO INTRANASAL INSTILLATIONS OF POLIOMYELITIS VIRUS IN NORMAL MONKEYS

	Sex	Number of monkeys	Result	
			Paralyzed	No symptoms
Stilbestrol	Female	3	3	0
	Male	2	2	0
Testosterone	Female	3	3	0
	Male	2	1	1
Stilbestrol and Testosterone	Female	3	2	1
	Male	2	2	0
No hormone	Female	3	1	2
	Male	2	2	0

all 5 monkeys became paralyzed; Group 2 (testosterone) 3 female and 1 male monkey came down with the disease; Group 3 (stilbestrol and testosterone) 2 female and 2 male animals became paralyzed; Group 4 (controls) 1 female and 2 male monkeys also developed poliomyelitis (Table 1).

EXPERIMENT 2

Twenty immature castrate monkeys, male and female, were divided into lots of 5 animals and subjected to the same hormonal procedures as in Experiment 1. This experiment was the same, except that all animals in Experiment 2 were castrated 2 weeks before beginning hormone injections. Intranasal instillations were begun on the tenth day after the first hormone injection, and repeated 5 times weekly until the first animal developed symptoms. Hormone injections were continued throughout the duration of the experiment.

External sexual changes present in all injected animals were indicative of activity of the hormone preparations. In Group 1 (stilbestrol) 1 male and 1 female monkey developed poliomyelitis; Group 2 (testosterone) all 5 monkeys came down with the disease; Group 3 (stil-

bestrol and testosterone) 1 female succumbed; Group 4 (controls) 2 male and 2 female monkeys became paralyzed.

The stilbestrol treated animals which had resisted intranasal instillation of virus were kept on hormone treatment and later, with 6 normal monkeys as controls, were inoculated intracerebrally with 0.6 cc. of a 5 percent emulsion of monkey poliomyelitis spinal cord. An interval of 20 days, between the time the last animal developed the disease from intranasal infection and the intracerebral inoculation, was allowed so that there would be no confusion as to which route of infection resulted in the attack. All intracerebrally inoculated monkeys developed the disease within the usual incubation period (Table 2).

TABLE 2. EFFECT OF STILBESTROL AND TESTOSTERONE ON RESISTANCE TO INTRANASAL INSTILLATION AND INTRACEREBRAL INOCULATION OF POLIOMYELITIS VIRUS IN CASTRATE MONKEYS

	Sex	Number of monkeys	Result—Intranasal		Result—Intracerebral	
			Paralyzed	No symptoms	Paralyzed	No symptoms
Stilbestrol	Female	3	1	2	2	0
	Male	2	1	1	1	0
Testosterone	Female	3	3	0	—	—
	Male	2	2	0	—	—
Stilbestrol and Testosterone	Female	3	1	2	2	0
	Male	2	0	2	2	0
No hormone	Female	3	2	1	*4	0
	Male	2	2	0	*3	0

* 3 normal monkeys added as virus controls.

EXPERIMENT 3

Six castrate female monkeys were given 0.1 mg. stilbestrol intramuscularly 3 times weekly for one week. The dose was then reduced to 0.05 mg. given 3 times weekly throughout the duration of the experiment. The effectiveness of the hormone was indicated by typical external changes. Following preparatory treatment of 24 days duration these monkeys, together with 6 untreated castrate females, were given an intranasal instillation of 2 drops of a 5 percent emulsion of monkey poliomyelitis spinal cord. The virus instillation was repeated each time the animals received a stilbestrol treatment. The first four instillations were of 2 drops each, then throughout the remainder of the experiment the dose was increased to 6 drops. At the end of a 50 day period, during which 19 virus instillations had been given, 5 of the 6 untreated castrate monkeys had developed poliomyelitis, while 2 of the 6 treated monkeys had come down with the disease.

The surviving treated monkeys were kept on stilbestrol treatment

and 27 days after the last monkey became infected following intranasally instilled virus, were, together with the surviving control animal, inoculated intracerebrally with 0.6 cc. of a 5 per cent emulsion of the virus. All intracerebrally inoculated animals developed typical experimental poliomyelitis.

The results of Experiments 1, 2 and 3 are summarized in Table 3. Since sex of the animal apparently had no influence on the outcome of these experiments the results are summarized in this table without regard to sex. It will be noted that stilbestrol alone or in combination with testosterone results in an increased resistance in the castrate host to intranasal instillations of the virus. Testosterone alone had no protective effect, while neither hormone increased resistance in the normal monkey. The most striking difference is between the stilbestrol

TABLE 3. A SUMMARY OF EXPERIMENTS 1, 2 AND 3, COMPARING THE EFFECT OF STILBESTROL AND TESTOSTERONE ON RESISTANCE TO INTRANASAL INSTILLATION OF POLIOMYELITIS VIRUS IN NORMAL AND CASTRATE MONKEYS AND ON RESISTANCE TO INTRACEREBRAL INOCULATION IN THE CASTRATE

	Monkey	Number of monkeys	Result—Intranasal		Result—Intracerebral	
			Paralyzed	No symptoms	Paralyzed	No symptoms
Stilbestrol	Normal	5	5	0	—	—
	Castrate	11	4	*7	6	0
Testosterone	Normal	5	4	1	—	—
	Castrate	5	5	0	—	—
Stilbestrol and Testosterone	Normal	5	4	1	—	—
	Castrate	5	1	4	4	0
No hormone	Normal	5	3	2	—	—
	Castrate	11	9	2	**8	0

* 1 monkey died of intercurrent disease 4 days before intracerebral inoculations.
** 6 normal monkeys added as virus controls.

treated castrate with an attack rate of 31 per cent and the untreated control castrate with an attack rate of 81 per cent.

That repeated intranasal instillations of virus under the protection of stilbestrol did not serve to immunize is indicated by the fact that subsequent intracerebral inoculation resulted in experimental poliomyelitis in all animals so treated. That the intracerebral inoculations were not too severe a challenge as a test for immunity is suggested by resistance of intracutaneously immunized monkeys to similar intracerebral inoculations (Aycock and Kagan, 1927).

SUMMARY

Experiments are reported which serve to emphasize the set of conditions under which monkeys exhibit increased resistance to experi-

mental poliomyelitis under estrogenic hormone as previously reported. Treatment of normal monkeys with stilbestrol produced no increase in resistance, while treated castrates showed a marked increase in resistance to intranasal instillations of the virus. Stilbestrol treated animals which had resisted repeated intranasal instillations of virus although kept on stilbestrol showed no increased resistance to subsequent intracerebral inoculation.

REFERENCES

ANDERSON, J. A., AND V. BOLIN: *Endocrinology* 39: 67. 1946.
ARMSTRONG, C., AND W. T. HARRISON: *Pub. Health Rep.* 51: 203. 1936.
AYCOCK, W. L., AND J. R. KAGAN: *J. Immunol.* 14: 85. 1927.
AYCOCK, W. L.: *Am. J. Pub. Health.* 27: 575. 1937.
AYCOCK, W. L.: *Endocrinology* 27: 49. 1940.
FOLEY, G. E., AND W. L. AYCOCK: *Endocrinology* 37: 245. 1945.
FRANCIS, T., C. E. KRILL, J. A. TOOMEY AND W. N. MACK: *J.A.M.A.* 119: 1392. 1942.
KESSEL, J. F., AND F. J. MOORE: *Am. J. Hyg.* 41: 25. 1945.
KRAMER, S. D.: *Proc. Soc. Exper. Biol. & Med.* 32: 1165. 1935.
MOORE, F. J., AND J. F. KESSEL: *Am. J. Hyg.* 38: 323. 1943.
SABIN, A. B., P. K. OLITSKY AND H. R. COX: *J. Exper. Med.* 63: 877. 1936.
SCHULTZ, E. W., AND L. P. GEBHARDT: *Soc. Proc. Exper. Biol. & Med.* 34: 133. 1936.
SCHULTZ, E. W.: *Proc. Soc. Exper. Biol. & Med.* 48: 135. 1941.
TRASK, J. D., A. J. VIGNEC AND J. E. PAUL: *Proc. Soc. Exper. Biol. & Med.* 38: 147. 1938.

THE EFFECTS OF INSULIN AND ANTERIOR PITUITARY EXTRACT ON THE BLOOD AMINO NITROGEN IN EVISCERATED RATS[1]

ELIZABETH G. FRAME[2] AND JANE A. RUSSELL

From the Department of Physiological Chemistry, Yale University School of Medicine

NEW HAVEN, CONNECTICUT

IT IS well recognized that the intact animal maintains the concentration of blood amino acids at a fairly constant level and that in only very few circumstances do the values vary significantly from normal. Since the liver is the chief organ of deamination in the body, it seemed that observations on the blood amino acid levels in the liverless animal should afford a good opportunity for a study of the regulation by different agents of amino acid metabolism.

The first successful hepatectomies were reported in dogs by Mann (1921); and Bollman, Mann and Magath (1926) showed that such an operation was followed within a few hours by a marked rise in the blood amino acid nitrogen level. This observation has been confirmed for the monkey by Maddock and Drury (1938), and for the rabbit by Svedberg, Maddock and Drury (1938). The effects of evisceration on blood amino acid levels were studied by Svedberg *et al.* (1938) in the rabbit, and by Mirsky (1938) in the dog, and in both studies the values were found to reach higher levels than in hepatectomized animals. The present paper gives the first detailed report on changes in blood amino acid nitrogen in the rat after evisceration.

EXPERIMENTAL

Albino male rats of the Sprague-Dawley strain, weighing 150–225 gms., were used. After a 24-hour fast they were eviscerated in a single-stage operation according to the procedure outlined by Russell (1942). Such an animal retains its liver but the hepatic blood supply has been cut off, so that the result in a "functional" hepatectomy as indicated by the finding that the total carbohydrate content of the liver is essentially unchanged during the period succeeding the operation. Immediately following evisceration the animals were given glucose to maintain the blood sugar level. This was ad-

Received for publication September 12, 1946.

[1] Part of the material in this paper was included in a thesis presented in 1942 by Elizabeth G. Frame to the faculty of the Graduate School, Yale University, in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

[2] Present address: Department of Physiological Chemistry, The Medical School, University of Minnesota, Minneapolis, Minnesota.

ministered either by subcutaneous injection of 100 mg. per 100 gm. body weight as a 10 per cent solution, or by constant intravenous infusion of 15 mg. in saline per 100 gm. body weight/hour.

In the experiments involving the use of insulin the hormone was given as a solution of zinc insulin crystals (Lilly). In 6 experiments, 1.0 unit per 100 gm. body weight was given subcutaneously immediately after evisceration, followed by 400 mg. glucose per 100 gm. body weight. Of these rats, 5 developed hypoglycemic convulsions within 3 to 4 hours and died. In 2 animals the amount of insulin was reduced to 0.5 unit, with the same amount of glucose as before, and here convulsions did not occur. Seven rats were infused intravenously with glucose and insulin together, the proportions of the 2 compounds being adjusted to avoid hypoglycemia; 0.2 unit insulin and 50–60 mg. glucose per 100 gm. body weight per hour were found to result in blood sugars closest to normal levels.

The pituitary extract used was a crude saline extract of beef pituitary glands, and was kept frozen until shortly before use. One ml. of the extract represented 200 mg. of the original gland. Amounts varying between 2 and 4 ml. were administered subcutaneously to rats during periods of 1 to 6 hours prior to evisceration. Treatment with glucose with or without insulin was similar to that already described.

The animals remained under nembutal anaesthesia throughout the experiment. Samples of blood were drawn from the tail for amino nitrogen determinations immediately after evisceration and at hourly intervals thereafter for as long as the animal survived. The survival period varied considerably, but in most cases was from 6–8 hours, with 9 hours in 1 animal being the longest survival period attained. The final blood sample in each case was taken from the heart after respiration had ceased but while the heart was still beating. Sugar determinations were performed in some experiments on the final blood sample.

Blood amino nitrogen determinations were made according to the method of Frame *et al.* (1943); blood sugar according to the procedure of Somogyi (1937), on copper-tungstate filtrates; and plasma uric acid by the method of Benedict (1922).

RESULTS

Evisceration

Table 1 and Figure 1 illustrate the effects of evisceration on whole blood amino acid nitrogen levels. Of the 17 animals in this series, 14 were given glucose subcutaneously while the remaining 3 were given constant intravenous infusions of glucose. The results with the 2 procedures agreed so closely that the data have been combined. The grouping of the data under intervals of time in the later periods after evisceration was required by the fact that the final samples were obtained at irregular intervals. From the initial mean of 15 mg. per cent, the nitrogen rose steadily to twice this value in 4 hours and to 45 mg. per cent in 6 hours. In 3 animals surviving longer than 8 hours a mean value of 65 mg. per cent was reached, with a value of 76 mg. per cent in 1 case. Figure 1 shows that the rate of rise increased gradually up to 3 hours and that after this time there was an increase

TABLE 1. EFFECTS OF EVISCERATION, INSULIN AND APE† ON BLOOD AMINO NITROGEN* IN RATS

Treatment	No. of Experiments	Hours after Evisceration 0	2	3	4	4½–5½	5½–6½	6½–7½	7½–8	>8
					A. ABSOLUTE LEVELS					
Evisceration	17	15.1 ±0.26	19.0 ±0.31	23.3 ±0.98	29.7 ±1.15	38.1 ±1.7	43.3 ±2.1	51.2 ±2.9	58.4 ±2.9	65.0
Evisceration plus Insulin	7	15.3 ±0.55	16.0 ±0.38	17.2 ±0.69	19.4 ±0.45	24.0 ±0.94	28.1 ±0.90	32.7 ±0.14	40.3	
Evisceration plus APE	7	13.2 ±0.64	17.1 ±0.42		26.6 ±0.39	35.8	41.2	51.8		
Evisceration plus Insulin plus APE	6	15.0 ±0.30	14.1 ±0.17	14.7 ±0.50	15.8 ±0.30	18.3 ±1.1	19.6 ±0.39	24.2 ±0.76	30.5	29.3
					B. CHANGES FROM INITIAL LEVELS					
Evisceration	17		4.0 ±0.45	7.9 ±1.1	14.2 ±1.3	22.8 ±1.8	29.6 ±2.1	35.4 ±3.1	42.8 ±3.0	
Evisceration plus Insulin	7		0.64 ±0.64	1.1 ±0.38	4.0 ±0.45	9.8 ±1.0	13.4 ±1.3	18.2	23.8	
Evisceration plus APE	7		3.8 ±0.52		13.8 ±0.57	23.3	26.7	37.1		
Evisceration plus Insulin plus APE	6		−0.92 ±0.13	−0.47 ±0.89	0.74 ±0.67	3.3 ±1.1	4.8 ±0.49	9.3 ±0.64	15.7	

* Means and standard errors of the means are expressed in mg. per cent.
† Anterior pituitary extract.

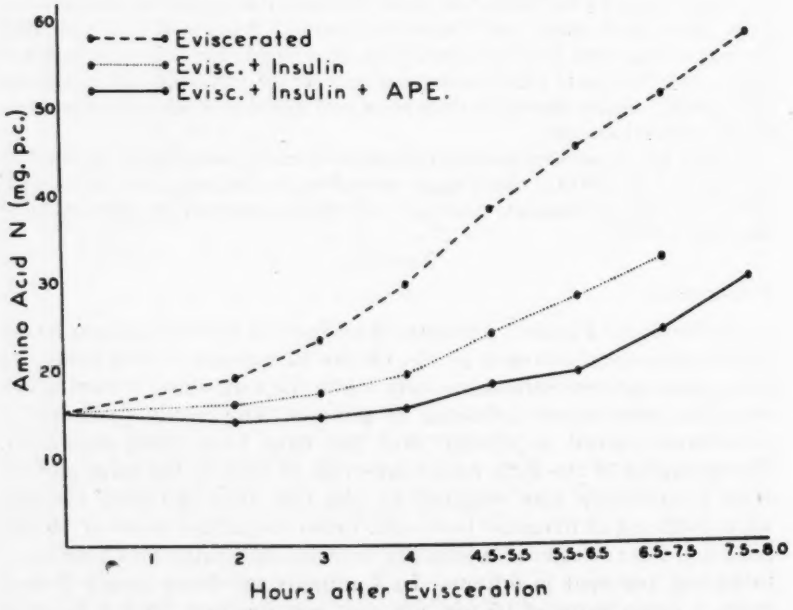


FIG. 1. The Effect of Insulin and Anterior Pituitary Extract (APE) on the Blood Amino Nitrogen in Eviscerated Rats.

to a rate which remained very nearly constant throughout the period. Data from the individual animals showed no relationship between survival time and either the rate at which the blood amino nitrogen rose or the amino nitrogen levels achieved. Terminal values obtained between 2 and 7 hours after evisceration were in general agreement with observations made at intermediate periods on animals surviving longer times.

In Table 1 the results are expressed also in terms of change from the initial level, in mg. per cent. This method of expression permits account to be taken of individual variations in the initial levels, which ranged from 13.5 to 17 mg. per cent and especially allows a more accurate comparison with the animals treated with pituitary extract, to be described later.

Plasma amino nitrogen levels were determined in 6 additional animals. Samples of blood were drawn from the tail at 2 and 4 hours after evisceration and in 1 instance also at 5 hours. In order to avoid further withdrawal of the relatively large amounts of blood necessary for determinations on plasma, all subsequent values were obtained from terminal heart blood samples. Starting from a mean value of 8.2 mg. per cent the average plasma amino nitrogen levels rose to 14, 26, 35, 49 and 60 mg. per cent in 2, 3, 4, 5, and 8 hours respectively. One animal which survived for 9 hours had a plasma value of 75 mg. per cent, equalling the highest value obtained on whole blood. In the initial samples the amino nitrogen concentrations were evidently lower in the plasma than in the cells, but at 4 hours or more after evisceration the plasma levels were within the range of values obtained on whole blood. Without information on plasma and whole blood values in the same animals, it is not possible to decide whether the increased amino nitrogen content is due to an increase chiefly in the plasma as Engel, Winton and Long (1943) found to be the case after hemorrhage, or to an increased concentration in both plasma and cells.

Glucose determinations were performed on 5 of the terminal blood samples, the values of 33, 65, 71, 76, and 91 mg. per cent being obtained. No relationship between blood sugar and blood amino acid levels was apparent.

The uric acid content of the plasma was determined in 5 animals. Starting from an initial value of about 2 mg. per cent the average uric acid level rose to 14 mg. in 4 hours, 28 mg. in 7 hours and 34 mg. in 8 hours after evisceration. The results indicate that uric acid is formed in the peripheral tissues, and accumulates in the blood when uricase, the enzyme of the liver which normally converts uric acid into allantoin, is lacking from the system. It has been shown by Frame *et al.* (1943) that in the procedure employed in the present study for the determination of amino acid nitrogen, 28 per cent of uric acid nitrogen, or about 9 per cent of uric acid, is determined as amino nitrogen.

Even the high levels of uric acid cited above do not interfere seriously with the values as given here for amino acid nitrogen, since they can have contributed only a few mg. per cent to the high amino nitrogen figures obtained in these experiments.

Insulin

The effects of the administration of insulin and glucose to the eviscerated rat are shown in Table 1 and Figure 1. Data on only the 7 animals which received glucose and insulin together by constant intravenous infusion are included. The results are qualitatively similar whether the compounds are administered by the subcutaneous or intravenous route, but in the former there is a slight time lag owing to the relative slowness of absorption of insulin. The effect of insulin in slowing the rate and extent of accumulation of amino acid nitrogen is marked, and the differences between the groups of animals with and without insulin are statistically significant for all time intervals after evisceration. Terminal blood sugar values showed marked individual variation. With the exception of 1 animal which had a value of 42 mg. per cent, hyperglycemic levels were observed, varying from 101 to 280 mg. per cent, but there was no correlation between the levels of glucose and of amino acid nitrogen in the blood.

Adrenal-demedullation

Five rats were adrenal-demedullated 3 to 4 weeks prior to evisceration and observation as described above. The whole blood amino nitrogen values tended to be slightly lower in the adrenal-demedullated animals as compared with animals having intact adrenals, both initially and in terms of rise above the initial level, but the figures were not markedly different in the 2 groups of animals. It may therefore be concluded that adrenal-demedullation has slight, if any, effect on the increase in blood amino nitrogen content. Two adrenal-demedullated animals were infused with glucose and insulin together. The results were in close agreement with those obtained from normal animals receiving similar treatment.

Anterior Pituitary Extract

The blood amino nitrogen levels were followed in 7 normal (non-eviscerate) rats given anterior pituitary extract (APE) during the course of 6 hours, and a progressive and statistically significant decrease from an initial mean value of 16.8 mg. per cent to 13.2 mg. per cent at the end of 6 hours was noted. At this time evisceration was performed and glucose was administered either subcutaneously or by constant intravenous infusion. The results, presented in Table 1, show very close agreement in the effects of evisceration on blood amino nitrogen levels with and without APE, indicating that the factor in APE which causes a lowering of the blood amino nitrogen in

the intact animal must act through some organ which was removed by evisceration. Additional amounts of extract were given to some of the animals at intervals after evisceration, demonstrating that the lack of effect in the eviscerated rat is not due to a wearing off of the action of the extract after 6 hours.

Nine rats were given APE over the course of 1 hour before evisceration. Of these, 3 received insulin and glucose subcutaneously after the operation, while the remaining 6 were infused intravenously with the 2 substances in the ratio previously found to be the most suitable for maintenance of blood sugar levels. Only the latter group is included in the data in Table 1 and Figure 1 in order that a more accurate comparison may be made with the similar group not receiving APE. The data indicate that the animals receiving APE in addition to insulin had lower blood amino nitrogen levels at all time intervals than those receiving insulin alone. At both the 2- and 3-hour intervals the mean values were still below the initial value, and in 1 animal the initial level had not been reached after 4 hours. After this time there was a slow rise in all instances. Statistical analysis of the results from the 2 groups of animals receiving insulin, with and without APE, showed that P at all time intervals was less than 0.01, indicating that the 2 groups differ significantly from each other. The terminal blood sugar values in the animals receiving insulin and APE varied between 68 and 218 mg. per cent.

Urine Examination

Samples of bladder urine were removed from a number of animals at the conclusion of the experiment and examined qualitatively. Heating of the urine with ninhydrin solution resulted in the development of a deep blue color, indicating the presence of amino acids. These are normally present only in traces in the urine. This observation indicates the continuation of kidney function in the eviscerated rat. In all urine specimens a flocculent precipitate formed immediately after withdrawal from the bladder, and increased in amount as the sample cooled. This precipitate gave a positive murexide test, indicating that it was uric acid, a result in keeping with the high blood levels of uric acid mentioned above. Mann and his collaborators (1925–33) have previously reported high blood and urinary levels or uric acid in hepatectomized dogs.

DISCUSSION

The effect of evisceration on the blood amino nitrogen levels in the rat scarcely requires comment other than to emphasize the magnitude and constancy in rate of rise. It may also be pointed out that even when the organism is presumably amply supplied with glucose to meet its energy requirements there is continuous breakdown of tissue protein.

The evidence in the present experiments that insulin influences protein metabolism even when hypoglycemia is avoided is in agreement with previous investigations. The depressing effect of insulin on blood urea and non-protein nitrogen in human beings was noted by Janney and Shapiro (1926); on blood urea and amino acids in rabbits by Tashiro (1926); on blood amino acids in rabbits, rats and humans by Luck, Morrison and Wilbur (1928); on blood amino acid nitrogen in dogs by Kerr and Krikorian (1929); and on blood urea and non-protein nitrogen in rats by MacKay *et al.* (1939). The converse effect has been reported by Luetscher (1942), who found high levels of plasma amino acids in patients with severe untreated diabetes mellitus. Mirsky (1938) found that insulin decreased the extent of the increase in non-protein nitrogen and amino acid nitrogen in the blood in eviscerated nephrectomized dogs. The preliminary report, without detail, by Levine *et al.* (1945) indicates comparable results in the eviscerated rat. The mechanism whereby insulin exerts its protein-sparing action is not known. That such action, in part at least, is a peripheral one is evident since it can be demonstrated in the absence of the liver. Kiech and Luck (1928) suggested that the decrease in blood amino acids may be due to an increase in their rate of catabolism; but that this is not the only mechanism is demonstrated in the present experiments and in those of Mirsky, since such a device would require the liver for deamination. Whether insulin promotes the synthesis of tissue protein from blood amino acids or inhibits its breakdown is not known. The former alternative is suggested by the observation of Mirsky (1938) that intravenous glycine disappeared more quickly from the blood of the eviscerated dog given insulin than in the control animal.

Our observation that adrenal-demedullation was without significant effect on the blood amino nitrogen levels of the eviscerated rat given insulin is not in agreement with the results of Davis and Van Winkle (1934), who found that insulin had no effect on the blood amino acid level of the adrenal-demedullated rabbit, or with those of Luck and Morse (1933), who attributed the blood amino nitrogen-lowering effect of insulin to epinephrine liberation. The fact that those observations were made on intact rabbits, while those reported here were made on eviscerated rats, may indicate either that a species difference exists, or, more likely, that different mechanisms were in play in the different circumstances.

It is well recognized that the administration of certain anterior pituitary extracts to animals causes growth, which is reflected in a retention of nitrogen. One manifestation of this action is the depression of the non-protein nitrogen of the blood, as shown in the dog by Teel and Watkins (1929) and Teel and Cushing (1930) and by Gaebler (1933) and others for non-protein nitrogen, urea and amino acids. Similar results were obtained on normal rats in the present work.

That insulin plays some part in the mechanism of action of the growth-promoting principle of the anterior pituitary was first indicated in the work of Mirsky (1939). He found that in the nephrectomized dog after either evisceration or pancreatectomy the administration of APE caused a greater, rather than a lesser, increase in the blood amino acids and non-protein nitrogen levels, as compared to the rise observed in control animals not receiving the extract. The subsequent pertinent literature has recently been fully reviewed by Young (1945) and will be only briefly summarized here. Young (1940; 1941; 1944) has found that the administration of APE to dogs may be either diabetogenic or growth-promoting, whereas in rats, which are refractory towards the diabetogenic action, the extracts are only growth-promoting. He considers the response to be dependent on the adequacy of pancreatic function. More recently Young (1945) has stated the view that the secretory activity of the pancreatic islets is an important factor in the nitrogen retention which may be observed simultaneously with the diabetogenic activity of pituitary extract. Gaebler and his collaborators (1941; 1942) observed that the administration of a pituitary preparation to depancreatized dogs receiving constant doses of insulin did not cause nitrogen storage as it did in normal animals, but that if the insulin dosage was increased nitrogen storage resulted.

Our observation that the administration of APE was without effect on the blood amino acid level, and presumably on muscle protein metabolism, in the eviscerated animal not supplied with insulin is not in agreement with that of Mirsky (1939), who concluded that the direct action of APE on muscle is one of stimulating protein catabolism. The present results substantiate the view that insulin is concerned with the nitrogen-retaining action of the anterior pituitary, but do not support the postulate that APE exerts its nitrogen-sparing effect only through a pancreotropic action as has been suggested or implied by Mirsky (1939), Young (1941) and Ogilvie (1945). In these experiments the pancreas was not present, so that no additional amount of insulin could have been put out under the influence of the APE. Moreover, insulin was being supplied in large amounts so that the maximum effect which it was capable of exerting on protein metabolism in these conditions was most probably already occurring. (This is indicated in the finding that the blood amino acid nitrogen level remained about the same whether 1 or 2 units of insulin per kg. body weight per hour were given.) The fact that APE caused a further decrease in the blood amino nitrogen level indicates that the effect observed here was independent of the amount of insulin present in the body above a certain undetermined minimum. Our results suggest that a synergistic action may exist between a principle of the anterior pituitary and insulin; that is, that the action of insulin is necessary at some stage in the protein synthesis stimulated by APE. Analogous relationships between hormones of the anterior pituitary and of the adrenal cortex

have been suggested by Russell (1940) and by Long, Katzin and Fry (1940). However, the existence also of a true pancreatrophic effect of APE is not precluded by the results of the present experiments.

Reference should be made to the finding of Engel, Winton and Long (1943) that extensive hemorrhage leading to shock in normal rats caused a marked increase in the blood amino nitrogen level. In their series of animals, which were bled slowly up to 3 or 4 per cent of the body weight, the levels rose with time to values approximating those found in animals which were eviscerated and given insulin and glucose in the present study. The possibility is raised that the evisceration *per se* was a shocking procedure and that the insulin had a non-specific effect on amino acid nitrogen levels through improving the status of the animal. Against such a thesis the following points may be raised: (1) The operation was usually accompanied by very little bleeding, and little difficulty was experienced in bleeding the animal from the tail throughout the course of the experiment. (2) Rats in which the extra-hepatic viscera were removed as in the present operation but in which the hepatic artery remained patent were observed in this laboratory by Engel, Harrison and Long (1944). In these animals, the blood amino nitrogen concentration remained very near the original level during the first 4 hours after the operation and then rose only very slowly during the remainder of the 7 hour observation period. Hence, the increase in blood amino nitrogen which was observed in the present experiments most probably resulted from the exclusion of the liver from the circulation and not from shock following the operation *per se*. (3) The administration of insulin had no beneficial effect in prolonging the survival period. It is possible that the constant rate of rise in blood amino nitrogen found subsequent to the 4-hour interval after evisceration, which was not prevented by insulin or insulin plus APE, may have been in part the result of approaching shock. If the changes which occur within the first 4 to 5 hours after evisceration only are considered to be quite independent of the shock phenomenon, they demonstrate the more clearly the effects of insulin and APE administration on the blood amino nitrogen levels.

SUMMARY

In eviscerated rats supplied with glucose the amino nitrogen content of the blood rose continuously through the periods of survival which were observed.

The administration of insulin in addition to glucose caused a significant decrease in the rate and extent of rise in the blood amino acid content. This effect was not related to the blood sugar level.

Adrenal-demedullation did not influence the action of insulin on the blood amino acid content of the eviscerated rat.

The administration of whole anterior pituitary extract decreased

the blood amino acid content of the normal rat but was without effect in the eviscerated rat.

The administration of insulin together with anterior pituitary extract had a greater effect in decreasing the rise in blood amino acid levels in the eviscerated rat than did insulin alone.

REFERENCES

BENEDICT, S. R.: *J. Biol. Chem.* 51: 187. 1922.
BOLLMAN, J. L., AND F. C. MANN: *Am. J. Physiol.* 104: 242. 1933.
BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH: *Am. J. Physiol.* 72: 629. 1925
BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH: *Am. J. Physiol.* 78: 258. 1926.
DAVIS, B. L., AND W. VAN WINKLE: *J. Biol. Chem.* 104: 207. 1934.
ENGEL, F. L., M. G. WINTON AND C. N. H. LONG: *J. Exper. Med.* 77: 397. 1943.
ENGEL, F. L., H. C. HARRISON AND C. N. H. LONG: *J. Exper. Med.* 79: 9. 1944.
FRAME, E. G., J. A. RUSSELL AND A. E. WILHELMI: *J. Biol. Chem.* 149: 255. 1943.
GAEBLER, O. H.: *J. Exper. Med.* 57: 349. 1933.
GAEBLER, O. H., AND H. W. GALBRAITH: *Endocrinology* 28: 171. 1941.
GAEBLER, O. H., AND A. R. ROBINSON: *Endocrinology* 30: 627. 1942.
JANNEY, N. W., AND I. SHAPIRO: *Arch. Int. Med.* 38: 96. 1926.
KERR, S. E., AND V. H. KRIKORIAN: *J. Biol. Chem.* 81: 421. 1929.
KIECH, V. C., AND J. M. LUCK: *J. Biol. Chem.* 78: 257. 1928.
LEVINE, R., T. A. TEXIDOR, A. L. ABRAMS AND S. SOSKIN: *Fed. Proc.* 4: 45. 1945.
LONG, C. N. H., B. KATZIN AND E. G. FRY: *Endocrinology* 26: 309. 1940.
LUCK, J. M., G. MORRISON AND L. F. WILBUR: *J. Biol. Chem.* 77: 151. 1928.
LUCK, J. M., AND S. W. MORSE: *Biochem. J.* 27: 1648. 1933.
LUETSCHER, J. A.: *J. Clin. Investigation* 21: 275. 1942.
MACKAY, E. M., R. H. BARNES AND H. C. BERGMAN: *Am. J. Physiol.* 126: 155. 1939.
MADDOCK, S., AND A. SVEDBERG: *Am. J. Physiol.* 121: 203. 1938.
MANN, F. C.: *Am. J. M. Sc.* 161: 37. 1921.
MIRSKY, I. A.: *Am. J. Physiol.* 124: 569. 1938.
MIRSKY, I. A.: *Endocrinology* 25: 52. 1939.
OGILVIE, R. F.: *J. Endocrinology* 4: 152. 1945.
RUSSELL, J. A.: *Am. J. Physiol.* 128: 552. 1940.
RUSSELL, J. A.: *Am. J. Physiol.* 136: 95. 1942.
SOMOGYI, M.: *J. Biol. Chem.* 117: 771. 1937.
SVEDBERG, A., S. MADDOCK AND D. D. DRURY: *Am. J. Physiol.* 121: 209. 1938.
TASHIRO, K.: *Tohoku J. Exper. Med.* 7: 268. 1926.
TEEL, H. M., AND H. CUSHING: *Endocrinology* 14: 157. 1930.
TEEL, H. M., AND O. WATKINS: *Am. J. Physiol.* 89: 662. 1929.
YOUNG, F. G.: *Endocrinology* 26: 345. 1940.
YOUNG, F. G.: *Brit. M. J.* 2: 897. 1941.
YOUNG, F. G.: *Brit. M. J.* 2: 715. 1944.
YOUNG, F. G.: *Biochem. J.* 39: 515. 1945.

NOTES AND COMMENTS

THE EFFECT OF GONADOTROPHINS ON EXPLANTED FRAGMENTS OF OVARIES FROM IMMATURE MICE

A MORPHOGENETIC influence of anterior pituitary explants on fragments of testis from immature rats in vitro has been described by Gaillard and Varossieau (1940). The question was raised as to whether the morphological structure of immature ovaries of mammals, too, could be influenced in vitro by explanted tissue from the anterior lobe of the hypophysis or by adding gonadotrophins to the culture media.

A. 100 Fragments of ovaries (each about $\frac{1}{6}$ to $\frac{1}{8}$ part of an ovary) taken from mice (weight 6–8 gm.) were cultivated in media containing 75% of plasma (adult fowl), $12\frac{1}{2}$% of embryonic juice (chicken) and $12\frac{1}{2}$% of a physiologic solution (coagulum method). Another 100 fragments were cultivated in media containing 50% of serum (adult fowl) and 50% of a physiologic solution (liquid media).

In both media the aspect of the ovarian explants after cultivating for 4–9 days did not indicate that those media had exerted any morphogenetic influence. Degeneration phenomena occurred in the centre of the explants after about a week in vitro.

B. In the culture media mentioned above 150 ovarian explants were cultivated for 4–8 days, together with fragments of anterior pituitary tissue taken from mice and rats of different ages and sex (confronted cultures). Media in which anterior pituitary tissue has been cultivated have been shown to contain gonadotrophins, extracted from or produced by the explants (Cutting and Lewis, 1938).

The hypophyseal explants in our experiments were about half the size of the ovarian explants. Number of hypophyseal explants per ovarian explant: 1–3, distance hypophyseal explant-ovarian explant: 0–0.5 mm. In the series of experiments anterior pituitary tissue was taken from: male and female mice, weight: 6–8 gm., female mice, weights: 12, 15, 18, 25 and 30 gm. (non-pregnant) and adult male rats.

Contrary to the behavior of testis explants in these circumstances, even when the anterior pituitary tissue was adjacent to the follicles no morphogenetic influence could be demonstrated.

C. Commercial pituitary gonadotrophins (ambinon-Organon and preloban-Bayer) were added to the culture media in different concentrations, ranging from 10 to 0.00005 R.U. per cc. of the media. Number of ovarian explants: 140.

Commercial chorionic gonadotrophins (pregnyl-Organon and prolan-Bayer) were added to the culture media in different concentrations, ranging from 60 to 0.06 R.U. per cc. of the medium. Number of ovarian explants: 70. In half of this series of experiments anterior pituitary tissue, taken from mice, weight: 6–8 gm., was cultivated together with the ovarian explants.

Received for publication July 1, 1946.

After cultivating for 4–8 days the morphological structure of the explants proved to be the same as the morphological structure of the explants cultivated in the control media. This does not apply to the highest concentrations: here severe degeneration phenomena occurred rapidly, due to the fact that the commercial gonadotrophins used contained tricresol.

The contrast between the behaviour of explanted fragments of ovaries, taken from mice (weight 6–8 gm.) and the reaction in vivo of intact ovaries in this age group is striking. However, about the cause of this difference the experiments are not conclusive. The experiments of Podleschka and Dworzak (1934) suggest that marked differences between the response of the ovary to gonadotrophins in situ and in transplants depend largely on the absence of an adequate autonomous innervation in the latter. This factor may also account for the differences between the response in situ and in vitro.

SUMMARY

Contrary to the behavior of explants from immature testis, no morphogenetic influence of explanted anterior pituitary tissue on explants from immature ovaries could be demonstrated. The morphological structure of ovarian explants cultivated for 4–8 days in media to which commercial pituitary or chorionic gonadotrophins had been added proved to be the same as the morphological structure of the explants cultivated in the control media.

C. FRANCKE

From the Department of Tissue Culture
Histological Laboratory State University of Leiden,
Leiden, Holland

REFERENCES

CUTTING, W. C., AND M. R. LEWIS: *Arch. f. exper. Zellforsch.* 21: 523. 1938.
GAILLARD, P. J., AND W. W. VAROSSIEAU: *Arch. f. exper. Zellforsch.* 24: 141. 1940.
PODLESCHKA, K., AND H. DWORZAK: *Arch. f. Gynäk.* 155: 381. 1934.

NEW BOOK

THE HUMAN FRONTIER. By Roger J. Williams. viii+314 pp. $3.00. Harcourt, Brace and Company, New York, 1946.

The Human Frontier deals with the problem of Science and Society. An opening chapter entitled "Why the Science of Humankind" outlines the argument that better social control requires basic study of every phase of man's life and activity. "Only by learning its basic truths, teaching them to our youth, and by extending greatly the boundaries of our knowledge can we cope with numerous social problems: education, marriage, health, employment, charlatanism in politics and elsewhere, crime, alcoholism, group bigotry (whose name is legion), and war."

Few will disagree with this thesis, and fewer still will be uninterested in practical methods to bring about improvements in all these aspects of social behavior. "There is an acute need for scientific study of human beings directed along the lines suggested in the body of this book. This study will be of a practical nature and have as its purpose the development of all possible information and insights that will make social control possible."

This proposed study, if I understand it correctly, would seek exhaustively to describe and to establish the basis of individuality. Individual variations of metabolism, of the special senses, of endocrine activity and of psychological traits are presented in a series of chapters. The conclusion seems to be that each individual differs somewhat from every other person, living or dead. The implications of this conclusion are discussed in a further series of chapters dealing with tolerance, education, marriage, criminology, medicine and medical research, heredity and environment, leadership, employment and international relations. Throughout these chapters runs the recurring theme that lack of recognition of individual peculiarities causes intolerance, crime, divorce and war. This lack of understanding stems, in Dr. William's opinion, from the proclivity of scientists to deal with "Man-in-the-abstract," a statistical Mr. Average Man whose characteristics are so far from those of real persons that we are led astray.

This reviewer certainly will not debate the premise that real people are endowed with special characters, nor even that life would be better if this thought were more universal. Nevertheless, it does seem that Dr. Williams spends an inordinate amount of time on the obvious. The basic notion that each individual is unique is certainly not new. Without going into the history of ideas, it may be said fairly that Christian doctrine has long and religiously maintained that there are essential personal dignities. Yet, intolerance, war, famine and pestilence are still with us. It may well be that recognition of individual dignity is a necessary condition for tolerance but history indicates that it is not a sufficient one.

Dr. Williams suggests repeatedly that the past failure of science to direct social development is caused by the lack of thoroughness with which human beings have been studied. We constantly expose ourselves to danger because we cannot comprehensively list our allergies. Only luck can account for suc-

cessful marriage unless we thoroughly understand the wellsprings of human behavior. Choosing a career intelligently is impossible without the information provided by a battery of aptitude tests. All human endeavor can only be on a trial and error basis unless first we assemble all possible information and then test every alternative. For example, at the end of the chapter on psychological traits there is a list of 25 questions, the answers to which " . . . taken together could form the basis of an intelligent approach to all one's future activities." Sample questions are: "Does my skin temperature adjust itself rapidly or slowly and does this have any bearing on my tendency toward colds?" "What are my requirements, relatively, for physical exercise?" "How am I distinctive in regard to the seven (or more) types of mental ability which people possess in varying degrees: spatial imagery, facility with numbers, word facility, etc? How should these aptitudes influence my life and work? (A large question.)"

A large question indeed. One wonders whether this brave new world of individuality may practically be attained after all. Perhaps the procedure suggested in this book would prevent future wars, but only because everyone would be furiously busy testing and being tested by everyone else.

These criticisms would be pointless if the general subject of Science and Society were less important. Recent events have forcefully indicated the need for a Science of Humankind. Several years ago, Dr. Walter B. Cannon wrote an inspiring essay entitled *The Body Physiologic and the Body Politic* in which he discussed the analogies between physiological homeostasis and stability of the social structure. The Harvard report on *General Education in a Free Society* considered the impact of science on our daily lives and educational methods. The Bush report, *Science, the Endless Frontier*, presents the case for rapid extension of scientific knowledge. A Conference held this year at Bar Harbor outlined methods of attack on fundamental social problems. Dr. William's book has the virtue that it carries the topic to the general public, but in view of the thoughtful contributions listed above, it is too bad that the problem has been so oversimplified.

EDWARD W. DEMPSEY

AUTHOR INDEX, VOLUME 39

ALBERT, A., W. L. MONEY AND M. X. ZARROW. Preparation of relaxin from sow corpora lutea residue, 270

ALBERT S., AND C. P. LEBLOND. The distribution of the feulgen and 2,4-dinitrophenylhydrazine reactions in normal, castrated, adrenalectomized and hormonally treated rats, 386

AYCOCK, W. L., see CURLEY, F. J., 414

BARKER, S. B. Effect of thyroid activity upon metabolic response to dinitro-ortho-cresol, 234

BELANGER, L. F., AND C. P. LEBLOND. A method for locating radioactive elements in tissues by covering histological sections with a photographic emulsion, 8

BIDDULPH, C., see MEYER, R. K., 23

BIRD, S. The influence of ingested estrogens on feed intake, metabolic rate and lipemia in male fowl, 149

BISSELL, A., see VANDERLAAN, W. P., 157

BOREL, H., see BRUZZONE, S., 194

BOYER, P. K., see DANFORTH, D. N., 188

BRUZZONE, S., H. BOREL AND J. SCHWARZ. The effect of steroids related to the cortical hormones and of stilbestrol on the adrenalectomized guinea pig, 194

CHAIKOFF, I. L., see WOLFF, J., 140

CLARK, G. Estrogens and the sex skin in the male chimpanzee, 155

COLE, H. H. Estrogen in late pregnancy mare serum and ovarian inhibition, 177

CRAFTS, R. C. Effects of hypophysectomy, castration, and testosterone propionate on hemopoiesis in the adult male rat, 401

CURLEY, F. J., AND W. L. AYCOCK. The effect of stilbestrol on resistance to experimental poliomyelitis, 414

DANFORTH, D. N., P. K. BOYER AND S. GRAFF. Fluctuations in weight, hematocrit and plasma protein with the menstrual cycle, 188

DEMPSEY, E. W. Book Review: The Human Frontier by Roger J. Williams, 432

DOUGHERTY, T. F., AND A. WHITE. Pituitary-adrenal cortical control of lymphocyte structure and function as revealed by experimental x-radiation, 370

DUNNING, WILHELMINA, F., see SEGALOFF, A., 289

ELMADJIAN, F., H. FREEMAN AND G. PINCUS. The adrenal cortex and the lymphocytopenia due to glucose administration, 293

EVANS, H. M., see INGLE, D. J., 32

EVANS, H. M., see SIMPSON, MIRIAM E., 281

EVANS, H. M., see SIMPSON, MIRIAM E., 286

EVERETT, J. W., AND C. H. SAWYER. Effects of castration and treatment with sex steroids on the synthesis of serum cholinesterase in the rat, 323

EVERETT, J. W. see SAWYER, C. H., 307

FINERTY, J. C., see MEYER, R. K., 23

FOGLIA, V. G., see HOUSSAY, B. A., 361

FRAME, ELIZABETH G., AND JANE A. RUSSELL. The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats, 420

FRANCKE, C. The effect of gonadotrophins on explanted fragments

of ovaries from immature mice, 430
FREEMAN, H., see ELMADJIAN, F., 293

GAEBLER, O. H., AND J. C. MATHIES. Further studies on effects of water soluble vitamins in experimental diabetes, 239
GRAFF, S., see DANFORTH, D. N., 188
GRIESBACH, W. E., see PURVES, H. D., 274

HART, G. H., S. W. MEAD AND W. M. REGAN. Stimulating the sex drive of bovine males in artificial insemination, 221
HARTMAN, C. G., J. L. LITTRELL AND J. TOM. The weanling guinea pig as test object for estrogen assays, 120
HISAW, F. L., see WRIGHT, P. A., 247
HOUSSAY, B. A., V. G. FOGLIA AND C. MARTINEZ. The influence of the thyroid on alloxan and pancreatic diabetes in the rat, 361
HUGGINS, C., AND P. S. RUSSELL. Quantitative effects of hypophysectomy on testis and prostate of dogs, 1

INGLE, D. J. The production of alimentary glycosuria by forced feeding in the rat, 43
INGLE, D. J., C. H. LI AND H. M. EVANS. The effect of adrenocorticotrophic hormone on the urinary excretion of sodium, chloride, potassium, nitrogen and glucose in normal rats, 32
INGLE, D. J., RUTH SHEPPARD, ELIZABETH A. OBERLE AND M. H. KUIZENGA. A comparison of the acute effects of corticosterone and 17-hydroxycorticosterone on body weight and the urinary excretion of sodium, chloride, potassium, nitrogen and glucose in the normal rat, 52

JUHN, MARY. Effect of thiouracil on the juvenile plumages of brown leghorn fowl, 14

KAZMIN, V., see VENNING, ELEANOR H., 131
KUIZENGA, M. H., see INGLE, D. J., 52
KURZROK, R., see LEONARD, S. L., 85
KURZROK, R., see LEONARD, S. L., 261

LACEY, A. H. Further observations on the rabbit method of insulin assay, 344
LEBLOND, C. P., see ALBERT, S., 386
LEBLOND, C. P., see BELANGER, L. F., 8
LEONARD, S. L., P. L. PERLMAN AND R. KURZROK. A turbidimetric method for determining hyaluronidase in semen and tissue extracts, 261
LEONARD, S. L., AND R. KURZROK. Inhibitors of hyaluronidase in blood sera and their effect on follicle cell dispersal, 85
LI, C. H., see INGLE, D. J., 32
LI, C. H., see SIMPSON, MIRIAM E., 286
LITTRELL, J. L., see HARTMAN, C. G., 120
LLOYD, C. W., W. F. ROGERS, JR., AND R. H. WILLIAMS. Quantitative studies of the opening of the vagina of immature rats following injection of female blood and urine, 256

MARTINEZ, C., see HOUSSAY, B. A., 361
MATHIES, J. C., see GAEBLER, O. H., 239
MCSHAN, W. H., see NALBANDOV, A. V., 91
MEAD, S. W., see HART, G. H., 221
MEYER, R. K., C. BIDDULPH AND J. C. FINERTY. Pituitary-gonad interaction in immature female parabiotic rats, 23
MEYER, R. K., see NALBANDOV, A. V., 91
MONEY, W. L., see ALBERT, A., 270

NALBANDOV, A. V., R. K. MEYER AND W. H. MCSHAN. Effect of purified gonadotropes on the androgen-secreting ability of testes of hypophysectomized cocks, 91
NIXON, W. L., see TIPTON, S. R., 300

OBERLE, ELIZABETH A., see INGLE, D. J., 52

PERLMAN, P. L., see LEONARD, S. L., 261
PHELPS, DORIS H. The experimental production of menstrual anomalies, 105
PINCUS, G., see ELMADJIAN, F., 293
PUGSLEY, L. I. The application of the principles of statistical analysis to the biological assay of hormones, 161
PURVES, H. D., AND W. E. GRIESBACH. The effect of thyroid administration on the thyrotropic activity of the rat pituitary, 274

RALLI, ELAINE P. Factors affecting survival in adrenalectomized rats, 225
REGAN, W. M., see HART, G. H., 221
ROBERTS, S., AND CLARA M. SZEGO. The nature of circulating estrogen: Lipoprotein-bound estrogen in human plasma, 183
ROGERS, JR., W. F., see LLOYD, C. W., 256
RUBIN, L., see WOLFF, J., 140
RUSSELL, JANE A. Book Review: Carbohydrate Metabolism by Samuel Soskin, 278
RUSSELL, JANE A., see FRAME, ELIZABETH G., 420
RUSSELL, P. S., see HUGGINS, C., 1

SAWYER, C. H., AND J. W. EVERETT. Effects of various hormonal conditions in the intact rat on the synthesis of serum cholinesterase, 307
SAWYER, C. H., see EVERETT, J. W., 323
SCHWARZ, J., see BRUZZONE, S., 194
SEGALOFF, A., AND WILHELMINA F. DUNNING. The inactivation of α-estradiol by inbred rats, 289
SHEPPARD, RUTH, see INGLE, D. J., 52
SIMPSON, MIRIAM E., C. H. LI AND H. M. EVANS. Absence of renotropic effects on the administration of pure adrenocorticotropic hormone (ACTH), 286
SIMPSON, MIRIAM E., AND H. M. EVANS. Comparison of the spermatogenic and androgenic properties of testosterone propionate with those of piruitary ICSH in hypophysectomized 40-day-old male rats, 281
SIMPSON, S. A., AND P. C. WILLIAMS. Increased pituitary weight produced by oestrone in intact and castrated rats, 272
SZEGO, CLARA M., see ROBERTS, S., 183

TAUROG, A., see WOLFF, J., 140
THOMPSON, K. W., Book Review: Encyclopedia of Endocrinology by Hans Selye, 224
TIPTON, S. R., AND W. L. NIXON. The effect of thiouracil on the succinoxidase and cytochrome oxidase of rat liver, 300
TOM, J., see HARTMAN, C. G., 120

VANDERLAAN, W. P., AND A. BISSELL. Effects of prophylthiouracil and of potasium thiocyanate on the uptake of iodine by the thyroid gland of the rat, 157
VENNING, ELEANOR H. Adrenal function in pregnancy, 203
VENNING, ELEANOR H., AND V. KAZMIN. Excretion of urinary corticoids and 17-ketosteroids in the normal individual, 131

WHITE, A., see DOUGHERTY, T. F., 370
WILLIAMS, P. C., see SIMPSON, S. A., 272
WILLIAMS, R. H., see LLOYD, C. W., 256
WOLFF, J., I. L. CHAIKOFF, A. TAUROG AND L. RUBIN. The disturbance in iodine metabolism produced by thiocyanate: The mechanism of its goitrogenic action with radioactive iodine as indicator, 140
WRIGHT, P. A., AND F. L. HISAW. Effect of mammalian pituitary gonadotropins on ovulation in the frog, *rana pipiens*, 247

ZARROW, M. X., see ALBERT, A., 270

SUBJECT INDEX, VOLUME 39

Abstracts
of papers presented at Annual Meeting, 58
Adrenal
relation to renal function, 32, 52
excretion of hormones of, 131
hormone effects, 194
function in pregnancy, 203
relation to survival, 225
and renal effects, 286
and lymphocytopenia, 293
relation to pituitary and lymphocytes, 370
and histochemistry of sex apparatus, 386
Alloxan
diabetes and relation to thyroid, 361
Androgens
secretion after gonadotropes, 91
stimulation by ICSH, 281
and cholinesterase, 323
and hemopoiesis, 401
Assay
of estrogens, 120
statistical methods in, 161
of estrogens in blood, 256
of thyroid hormone, 274
of insulin, 344
Association notices, 223, 280, 358

Corpora lutea
preparation of relaxin from, 270

Diabetes
of forced feeding, 43
effect of vitamins on, 239
relation to thyroid, 361

Enzymes
and thyroid, 300
hormones and cholinesterase, 307
castration and cholinesterase, 323
Estrogens
assay of by weanling guinea pig, 120
and metabolism of fowl, 149
and sex skin of chimpanzee, 155
and ovarian inhibition, 177
nature of in plasma, 183
effect after adrenalectomy, 194
assay of, 256
effects on pituitary, 272
inactivation of, 289
and cholinesterase, 323
and reslstance to poliomyelitis, 414

Glycosuria
caused by forced feeding, 43
Goitrogens
and iodine metabolism, 140
effect on iodine uptake in thyroid, 157
effect on enzyme systems, 300
Gonads
relation to pituitary, 23

Histochemistry
location of radioactivity in tissues, 8
of sex structures in rat, 386
Hyaluronidase
inhibitors in serum, 85
method of determining, 261
Hypophysectomy
effect on testis and prostate, 1
effects of gonadotropes after, 91

Insulin
and blood amino nitrogen, 420
Iodine
location of radioiodine, 1
disturbance of metabolism of, 140

Ketosteroids
excretion of, 120

Liver
enzyme system in, 300

Menstruation
anomalies of, 105
weight and blood changes during cycle, 188

New Books
Encyclopedia of Endocrinology, 224
Carbohydrate Metabolism, 278
The Human Frontier, 432

Ovary
effect of hyaluronidase on follicle cells, 85
inhibition by estrogens, 177
relation to pituitary in frog, 247
preparation of relaxin from, 270
and gonadotrophins, 430

Pancreas
relation to diabetes, 361
Parabiosis
Pituitary-Gonad interaction in, 23
Pituitary
relation to gonads, 23
relation to androgen secretion, 91
relation to ovulation in frog, 247
effect of estrogen on, 272
relation to thyroid, 274
effect on testis, 281
and renal effects, 286
relation to adrenal and lymphocytes, 370
and hemopoiesis, 401
relation to blood amino nitrogen, 420
relation to transplanted ovaries, 430
Plumage
effect of thiouracil on, 14
Pregnancy
adrenal function in, 203
Prostate
effect of hypophysectomy on, 1

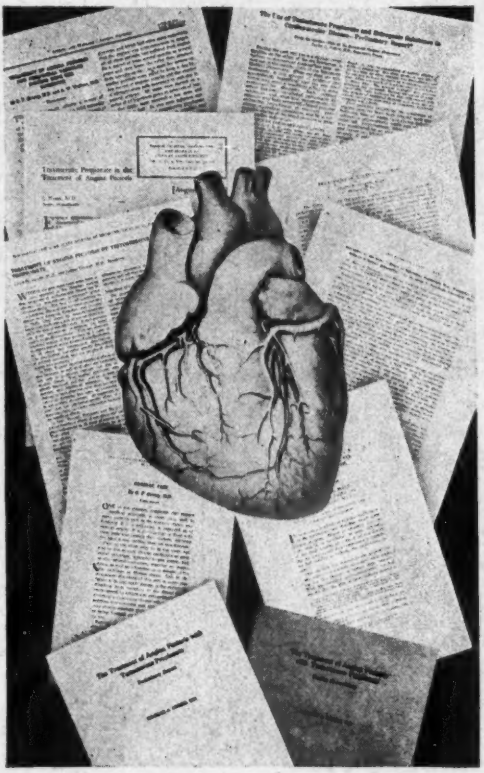
Radioactivity
location in tissues, 8
Relaxin
preparation from corpora lutea, 270

Semen
hyaluronidase content of, 261
Sex drive
stimulation of, 221

Testis
effect of hypophysectomy on, 1
effects of gonadotropes on, 91
relation to pituitary, 281
and hemopoiesis, 401
Thiouracil
effect on plumage in fowl, 14
effect on iodine uptake of thyroid, 157
effect on enzyme systems, 300
Thyroid
and plumage of fowl, 14
iodine metabolism of, 140
iodine uptake after goitrogens, 157
relation to dinitro-ortho-cresol, 234
relation to pituitary, 274
and enzyme systems, 300
relation to alloxan diabetes, 361

Vitamins
effects in diabetes, 239

Growing Evidence . . . of the Successful Use of Androgens in Angina Pectoris



CIBA BULLETIN No. 1
on
EXPERIMENTAL
HORMONE THERAPY

In the past few years a growing group of clinicians has been reporting gratifying results in the treatment of anginal patients with the androgen, testosterone propionate. Results have been noted after 6 to 8 weeks therapy.

Findings reported . . .

Exercise and emotional tolerance improved.[1]

Beneficial effects persisting 2 to 18 months.[2, 3]

The massing of favorable reports in this experimental indication warrants consideration and use of testosterone propionate in anginal syndrome.

PERANDREN . . .

MOST POTENT ANDROGEN

Available . . .

Ampuls 1 cc containing 5 mg.
Ampuls 1 cc containing 10 mg.
Ampuls 1 cc containing 25 mg.

Perandren — *Trade Mark Reg. U. S. Pat. Off.*
Denotes Ciba's Testosterone Propionate.

BIBLIOGRAPHY

(1) Walker, T. C.: Jl. Clin. Endocrinol., 1942, ii, 560-568.

(2) Lesser, M. A.: New England Jl. Med., 1942, ccxxvi, 51-54.

(3) Lesser, M. A.: New England Jl. Med., 1943, ccxxviii, 185-188.

CIBA

CIBA PHARMACEUTICAL PRODUCTS, INC., SUMMIT, NEW JERSEY

In Canada: Ciba Company Ltd., Montreal

HOW DO THE METABOLIC FIRES BURN?

In myxedema and cretinism, metabolic combustion is at a very low level. In lesser, subclinical or partial deficiencies physiologic oxidation is more rapid but still below normal. In minor as in major thyroid deficiencies, it is important to make certain that the thyroid medication employed is of good quality—potent, uniform and accurately standardized.

The name "ARMOUR" has long stood for "excellence and dependability" in thyroid medication . . . and with good reason. The Armour Laboratories has available the *world's largest supply of fresh raw material.* This is of fundamental importance. It makes practicable the selection and blending of animal glands in order to compensate for the regional and seasonal variation in natural thyroid. Armour was first to recognize this variation. Armour also was first to institute methods of assaying and blending the glands to fixed standards.

Rx *Thyroid Armour*

Supplied in 1/10, 1/4, 1/2, 1, 2 and 5 grain tablets, plain or sugar coated, and in powder, U. S. P.

Have confidence in the preparation you prescribe—specify "ARMOUR"

THE ARMOUR LABORATORIES • CHICAGO 9, ILLINOIS

HEADQUARTERS FOR MEDICINALS OF ANIMAL ORIGIN